Learning-dependent modulation of inhibitory transmission in hippocampal CA1 area of adult mice

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Preface

'The brain is like a muscle. When it is in use we feel very good. Understanding is joyous.'

----- Carl Sagan

Human brain is one of the most spectacular designs in nature. It is the brain that gives the human being the intellectual power to differ from other species. It is the brain that brings our ancestors through all kinds of challenges and difficulties to where we are today. It is as well the human brain that endows us full of emotions enabling us to communicate beyond words. After millions of years' evolution, our brain has developed into such an elegant organ with specific sets of genes to make it fit and in control of most of the needs in our life. It is what our specie experienced in the past hundreds of thousands of years determines how our brain works today.
In an individual's life, the same principle still applies. The genetic information is crucial for brain development and function, whereas life experience provides equally important impact on how brain works, if not more. Even people with same genetic background, like the identical twins, can think and behave in completely different ways in certain situations. Every single moment in our life, joyful or sad, inspiring or desperate, leaves its mark somewhere in our complicated neural labyrinth—the brain. It is our past life experiences define who we are, what we think and how we behave.

Everyone can feel the power of how past experiences shapes themselves, while neuroscientists try to understand in what ways the experience changes our mind. The brain is plastic and keeping changing throughout one's life. Neural plasticity has been intensively studied for decades, serving as a platform to understand our unique pattern of learning and memory. We now know that some forms of neural activity such as trains of action potentials in the neurons with specific frequencies, can lead to long-term change in the connections among neurons. Behaviourally, a subject can learn completely new tasks or link a neutral stimulus to

reward/punishment after certain training paradigms throughout the whole life. However, there are still gaps between how cellular plasticity and learning behaviour talk to each other in a fast and efficient way.

Works from our lab focus on understanding how learning and experience specifically influence neural circuit in hippocampus, a brain structure with a critical role in learning and memory; and how that plastic change in neural circuit in turn impacts on behaviour. Previous work by Donato et al. showed that behavioural learning could modulate the state of an inhibitory neural circuit, the parvalbumin (PV) interneurons network state in hippocampus. Contextual fear conditioning leads to a high-PV configuration, making further learning more difficult; while environmental enrichment leads to a low-PV configuration and makes further learning easier. How these PV interneurons work differently apart from the differential PV expressions after behavioural learning is largely unknown. It is intriguing to investigate whether and how PV interneurons function differently after behavioural learning and how the hippocampal neural circuit is affected by the plastic change in PV interneuron network. Meanwhile, there are subpopulations of principal cells in hippocampal network. Are these different subpopulations of principal cell affected in similar ways?

These questions constitute the main themes of my thesis work. By applying electrophysiological recording, immunostaining and behavioural training, I systematically examined the change of PV interneuron's intrinsic properties and its effect on hippocampal CA1 circuitry. It turns out that the intrinsic excitability of PV interneurons is modulated by previous experience, with a higher excitability after contextual fear conditioning and a complex change of excitability after environment enrichment. Intrinsic excitability reflects the propensity of a neuron to fire action potentials. Changes in intrinsic excitability can be more important than that in synaptic weight since it affects all the neuronal connections. The activity level of PV interneuron could determine the strength of inhibitory transmission and could further control learning tasks executed in hippocampus. This was examined by recording the inhibitory inputs in different subgroups of CA1 principal cells. The inhibitory inputs mediated by PV interneuron are differentially regulated by contextual fear conditioning and environment enrichment. These results provide the functional evidence that past experiences modulate the state of neural circuit underling learning through modulation of PV interneurons.

The search of secrets in brain never ends. We are still far from answering the ultimate questions, like how people get to fear or love, or how we facilitate the learning of beneficial experiences and erase the harmful impact by negative experience such as wars and natural disasters. I am proud of myself to be part of this challenging, and I am proud to contribute my own effort to the tremendously fascinating effort by all neuroscientists in the world.

Abstract

Learning not only shapes the brain to form new memories but also affects future learning. Previous studies show that Pavlovian contextual fear conditioning (cFC) and environmental enrichment (EE) can inhibit or facilitate further learning via shifting parvalbumin (PV) interneurons network towards a high PV or low PV state in hippocampal neural circuit, respectively (Donato et al., 2013). The aim of my thesis study is to reveal the physiological relevance of learning-induced changes in PV interneuron network, and to investigate how these changes affect information processing of excitatory microcircuits.

By combining transgenic mouse lines, electrophysiological and behavioural studies, we showed that cFC homogenized the firing behaviour of PV cells. More PV cells fired with no adaptation and higher firing rates; on the contrary, EE led to more diversified firing behaviour in PV cells: more cells fired with pronounced adaptation but within a wide range of firing frequency. Such learning-dependent intrinsic plasticity of PV cells further modified hippocampal CA1 inhibitory transmission. The results showed that the inhibitory transmission in CA1 area was enhanced upon cFC; similar to the modulation effect on intrinsic excitability, EE diversified its modulation on inhibitory transmission with different directions onto two sub-groups. Interestingly, when we examined the Lsi1 subpopulation of principal cells, cFC exerted equal modulation on PV cell-mediated inhibitory transmission in Lsi1 cells as in averaged group of pyramidal cells; while EE enhanced the total Inhibitory postsynaptic currents (IPSC) and slightly increased PV cell-mediated IPSCs, which is different from the averaged group of pyramidal cells in hippocampus.

Overall, these results suggest that the behavioural experience can differentially modulate inhibitory transmission in hippocampal CA1 with a subpopulation specific manner. This neurophysiological knowledge will facilitate our understanding on the information processing in experience-modified neural circuits and how such modulation influence further learning. Future studies will be required to examine the molecular mechanism supporting these changes in inhibitory circuits upon learning.

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Thanks for the support I got from my little daughter, my father and my sister.

At last, I would thank my beloved mother, for her providing me a safe growing environment and making few limitations on my way of growth.

Abbreviations

A	accommodating (spike frequency adaptation)
AMP	spike amplitude
AMPA	α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid
BDNF	brain-derived neurotrophic factor
CCK	Cholecystokinin
cFC	contextual fear conditioning
CS	conditioned stimulus
Cm	membrane capacitance
Ctrl	control
DG	dentate gyrus (hippocampal region)
EC	entorhinal cortex
EE	enviromental enrichment
fAHP	fast afterhyperpolarization
FFE	feed-forward excitation
FFI	feed-forward inhibition
FBI	feedback inhibition
FS	Fast-spiking
GABA	γ-amino butyric acid
GC	granule cell
I_h	hyperpolarization activated current
LMT	large mossy fibre terminal
LTD	long-term depression
LTP	long-term potentiation
MF	mossy fibres
mGFP	membrane-targeted green fluorescent protein
NA	non-accommodating firing pattern (spike frequency adaptation)
NMDA	N-methyl-D-aspartate
P	postnatal day
PV	Parvalbumin
Rm	membrane resistance
SA	strong accommodating firing pattern (spike frequency adaptation)
STF	short-term facilitation
STD	short-term depression
$t_{1/2}$	action potential half width

US	unconditioned stimulus
Vm	resting Membrane Potential
Vt	Firing threshold

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General Introduction

General Introduction

1.1 Hippocampal Formation

1.1.1 Hippocampal anatomy

The hippocampal formation, which includes the dentate gyrus (DG), the hippocampus proper (Cornu Ammonis regions CA3, CA2 and CA1) and the subiculum, is a complex brain structure that locates at the temporal lobe of the brain. The hippocampal formation is part of the limbic system. It plays important roles in the consolidation of memory from short-term memory to long-term memory and spatial navigation. Anatomy and connections between different sub-regions within the hippocampal formation are well reserved throughout all mammals.

As shown in Figure 1, the C-shaped rodent hippocampal formation is situated in the caudal part of a brain model. The longitudinal axis of hippocampal formation is called the dorsoventral (or septotemporal) axis, which runs from the dorsal pole via the intermediate to the ventral pole (or temporal, close to the amygdala). The dorsal and ventral parts are believed to serve different neural functions. (Bannerman et al., 2004; Goodrich-Hunsaker et al., 2008; Kesner et al., 2004)

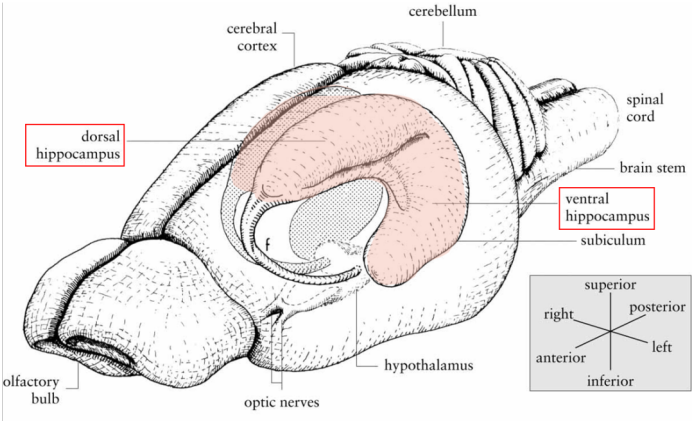

Figure 1: Three-dimensional representation of the rodent brain and the position of the hippocampal formation

Depiction of the rat brain and its main brain structures. Each hippocampus (highlighted in pale pink) is a C-shaped structure located in the caudal part of the brain. The top, anterior portion is

the dorsal hippocampus, while the caudal and inferior portion is the ventral hippocampus (red boxes). Three orientation axes are shown in the bottom right panel. Modified from (Amaral and Witter, 1995).

Hippocampus has distinguished features in term of its structure:

1. Lamination: Throughout the whole hippocampal formation (from dentate gyrus to subiculum), the cell body and fibres show clear layers, forming a laminated network. As shown in Figure2, in DG, CA3 and CA1, cell body of principal cell form a clear cell-body layer. Projection fibres in each part of hippocampus form their specific layers too.

2. Topography: Inputs from different origin located at specific layers form a precise topography map. Combined with the lamination, the topographical map of hippocampal formation provides an ideal structural basis for the functional dissection of a neural circuit.

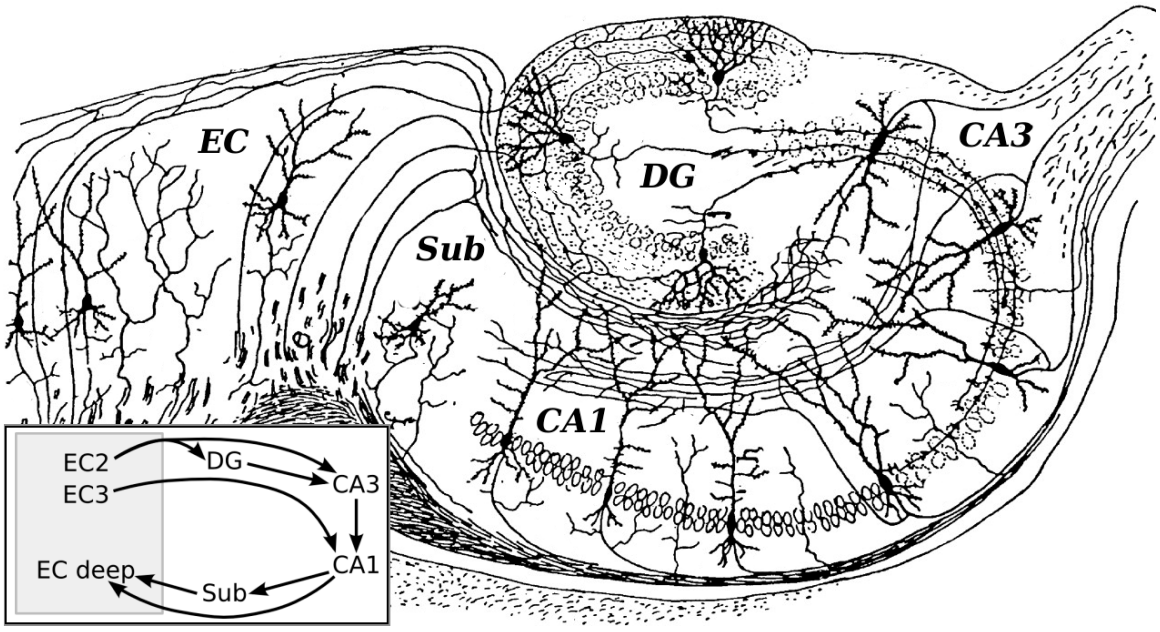

Figure 2: Modified drawing of the neural circuitry of the rodent hippocampus by Santiago Ramón y Cajal (1911)

Neural activity flows from entorhinal cortex (EC) to DG, then from DG to CA3. CA1 cells receive inputs from both CA3 and layer 3 of EC and project to subiculum and back to deep layers of EC. Cell body, axon fibres and apical dendritic tree of principal cells have their distinct layers.

1.1.2 Hippocampal functions

Memory

It is generally accepted that the hippocampus is important in the formation of new memories about past experience (episodic memory, Eichenbaum et al., 1993). Severe damage to the hippocampus could result in profound difficulties in forming new memories (anterograde amnesia). Memories that formed before the damage occurred (retrograde amnesia) are often affected too. The hippocampus damage does not affect some other types of learning and memory, such as the ability to learn new skills （Squire and Schacter，2002）.

Spatial memory and navigation

Studies on freely moving rodents have shown that many hippocampal neurons have "place fields": when the animal passes through a particular place of the environment, bursts of action potentials can be detected in these places cells. Pyramidal cells in the hippocampal CA1 and CA3 and granule cells in the dentate gyrus show such place cell responses. The discovery of place cells suggests that the hippocampus might act as a cognitive map. Animal studies have shown that an functional intact hippocampus is necessary for the initial learning and long-term retention of spatial memory, especially for tasks that require to find the way to a hidden goal (Morris et al., 1982).

Hippocampal functions in affective aspects

Besides the cognitive functions that hippocampus involved in, hippocampus can also function as a regulator of stress and emotions whose dysfunctions lead to psychopathology and affective disorders (Fanselow and Dong, 2010; Gray and McNaughton, 2000). The hippocampal functions in emotions are mainly attributed to its prominent position in Papez's limbic circuit, which regulate emotional responses (Papez, 1995). Besides, the hippocampus controls the hypothalamic-pituitary-adrenal axis, which controls stress hormones levels (Dedovic et al., 2009; Jacobson and Sapolsky, 1991). Consistently, elevations of corticosterone concentration are known to lead to hippocampal dysfunction (McEwen et al., 1997). Human studies showed that decreased hippocampal volumes and hippocampal dysfunctions are associated with psychopathologies such as posttraumatic stress disorder, bipolar disorder and depression (Bonne et al., 2008; Frey et al., 2007).

1.1.2 Subpopulations of principal cells in hippocampus

Although principal cells in hippocampus have often been tacitly assumed to be several major homogeneous populations (DG, CA3 and CA1), recent findings from our laboratory have demonstrated the existence of “microcircuits” in the hippocampus, consisting of subpopulations of genetically predefined principal neurons that are selectively interconnected as a result of temporally matched schedules of neurogenesis and synaptogenesis (Deguchi et al., 2011). Using sparse Thy1 mouse reporter lines (Lsi1 and Lsi2) based on a modified version of the Thy1.2 promoter cassette (Caroni, 1997), genetic sister neurons were shown to preferentially connect to each other across subfields (Figure 3).

Other studies show that there are subpopulations of principal cells which show distinct responses to inputs, spiking properties and output influence. They segregate in the superficial and deep parts of CA1 stratum pyramidal. Neurons residing in different sub-layers differed in neuronal properties such as theta phase preference and phase-modulation strength in REM sleep. Moreover, the modulation by slow oscillations, sharp-wave ripples during non-REM sleep and gamma phase preference during waking state can be heavily influenced by sub-layer location. Firing rate, bursting rate and the proportion of cells with place fields are also dependent on the sub-layer location (Mizuseki et al., 2012; Moser et al., 2011).

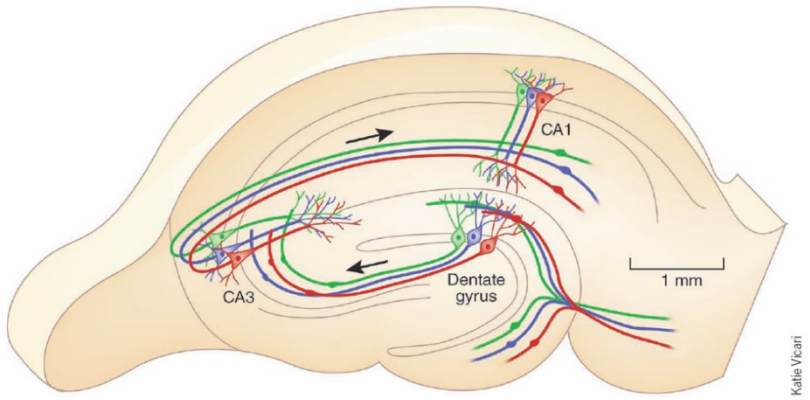

Figure 3: Parallel microcircuits in the hippocampus (Moser et al., 2011)

1.2 Inhibitory circuits in hippocampus

Among the studies of inhibition in neuron circuits, there are plenty of evidences showing that different types of interneuron selectively innervate different dendritic domains of pyramidal neurons. For examples, in the hippocampal CA1 region, the O-LM cells and bi-stratified cells only innervate the distal apical dendrite of CA1 pyramidal cells; whereas the basket cells selectively form synapses onto the peri-somatic region (Freund and Buzsaki, 1996; Parra et al., 1998; Somogyi et al., 1998). Research about differential shunting inhibition at different dendritic domain suggests the interneurons that innervate the proximal regions of target cell pyramidal cell soma like basket cells tend to have a global and nonselective effect on all the excitatory inputs, while the interneurons that project to innervate the distal dendrite of target cells like O-LM cells and bi-stratified cells selectively control the activity level of local dendrite domains and execute more sophisticated control on different pathways of excitatory input (Hao et.al., 2009).

1.2.1 Inhibitory transmission

Inhibition in central nervous system is mainly mediated by different GABA and glycine receptors. The inhibitory response can be hyperpolarizing or shunting, phasic or tonic. The details of the inhibitory response depend on the receptor subtypes. $GABA_A$ receptors predominantly localize opposed to inhibitory presynaptic GABAergic terminals (inhibitory synapses), but also outside of synapses (tonic inhibition). Similar to glutamatergic synapse, the GABAergic transmission can undergo activity-dependent plasticity and can be regulated by a wide range of neuromodulators.

1.2.2 Interneuron subtypes

In the hippocampus and cerebral cortex, many different types of interneurons together with principal cells, form intricate circuits and cooperates in time for the neural information computation. The description of functional connection of different interneurons reveals a spatiotemporal division of labour in the CA1 hippocampal area (Klausberger and Somogyi, 2008), as exemplified in Figure 4. Different interneurons can be classified by their specific peptide expression or their morphology. In hippocampus, different types of interneurons divide the surface of principal cells and fire action potentials in distinct time window, innervate either the

same or different subcellular compartments. The CCK and PV positive interneurons constitute the majority of basket cell, which selectively innervate the perisomatic region of pyramidal cells. In contrast, O-LM cells selectively innervate the distal dendrite (Figure 4). They also support distinct temporal dynamics of neural activity, such as network oscillations, selection of cell assemblies, and the implementation of brain states. The spatiotemporal arrangements in neural circuits reveal that cellular diversity and temporal dynamics co-emerged during evolution, providing a basis for cognitive behaviour.

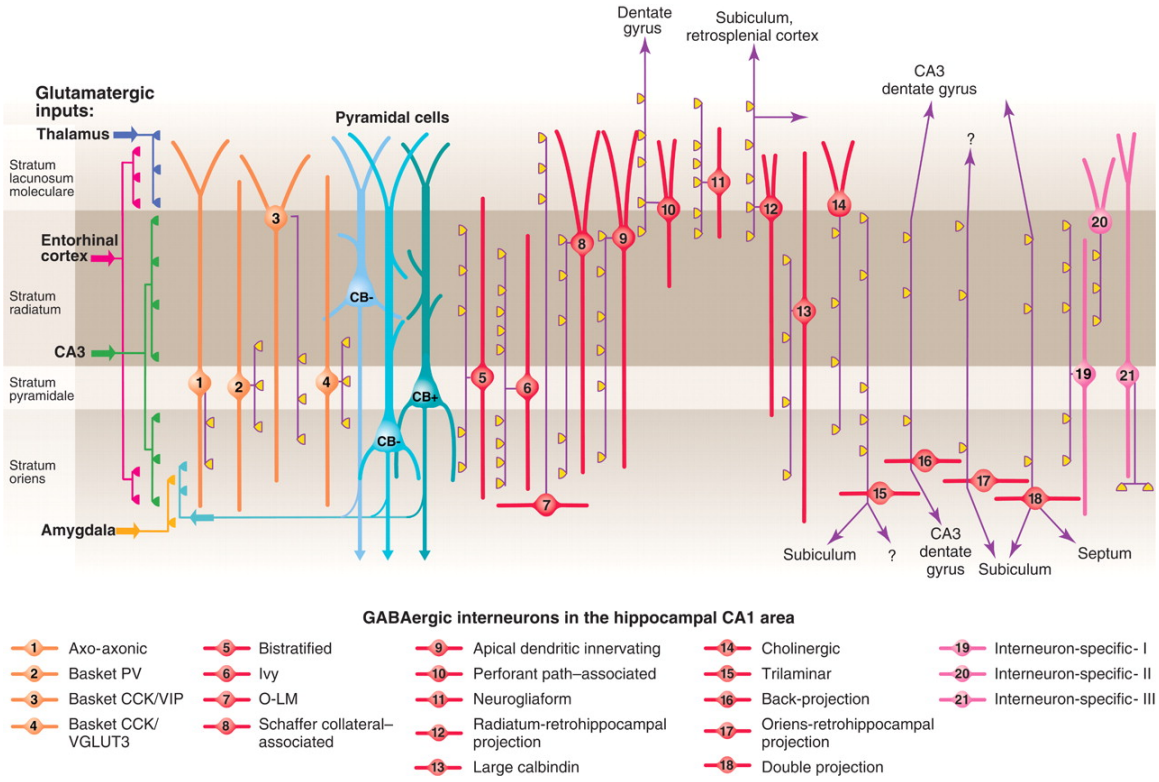

Figure 4: Different cell types in hippocampal CA1 region

Three types of pyramidal cell are accompanied by at least 21 classes of interneuron in the hippocampal CA1 area. The main terminations of five glutamatergic inputs are indicated on the left. The somata and dendrites of interneurons innervating pyramidal cells (blue) are orange, and those innervating mainly other interneurons are pink. Axons are purple; the main synaptic terminations are yellow. Note the association of the output synapses of different interneuron types with the perisomatic region (left) and either the Schaeffer collateral/commissural or the entorhinal pathway termination zones (right), respectively. VIP, vasoactive intestinal polypeptide; VGLUT, vesicular glutamate transporter; O-LM, oriens lacunosum molecular.

1.2.3 Parvalbumin-positive interneurons

During development, PV interneurons mainly derive from medial ganglionic eminence (MGE, Figure 5). Transcription factor Dlx5 in particular is expressed in the mature PV-positive interneuronal subset (Wang et al., 2010). By using transplantation experiments, Wang et al. demonstrate that loss of Dl×5 or both Dl×5 and 6 in mice lead to a significant reduction in the number of PV positive interneurons. It also leads to alteration in dendritic morphology, and epilepsy. The PV expressing interneurons constitute about 40% of the GABAergic cortical interneuron population (Rudy et al., 2011). The features of PV positive interneuron population are fast-spiking pattern, sustained firing, high-frequency trains and brief action potentials (Gibson et al., 1999). Additionally, among all interneurons, PV interneuron is of the lowest input resistance with the fastest membrane time constant (Ascoli et al., 2008). PV cells receive strong excitatory inputs from thalamus and cortex, as well as inhibition from other PV cells. They play an important role in stabilizing the activity of cortical networks. The absence of inhibition from PV cells can lead to epileptic form activity, while chronic dysfunction of PV interneurons has been shown to be involved in diseases such as schizophrenia. In case the PV-positive interneurons cannot function properly, the inhibitory input onto pyramidal neurons may be interrupted and synchronization in the gamma range is impaired (Lisman et al., 2008). This result is corroborated by observation that when schizophrenic patients were asked to do working memory tasks, they displayed abnormal gamma frequency oscillations in the prefrontal cortex, compared with healthy, non-schizophrenic subjects (Kelsom and Lu, 2013).

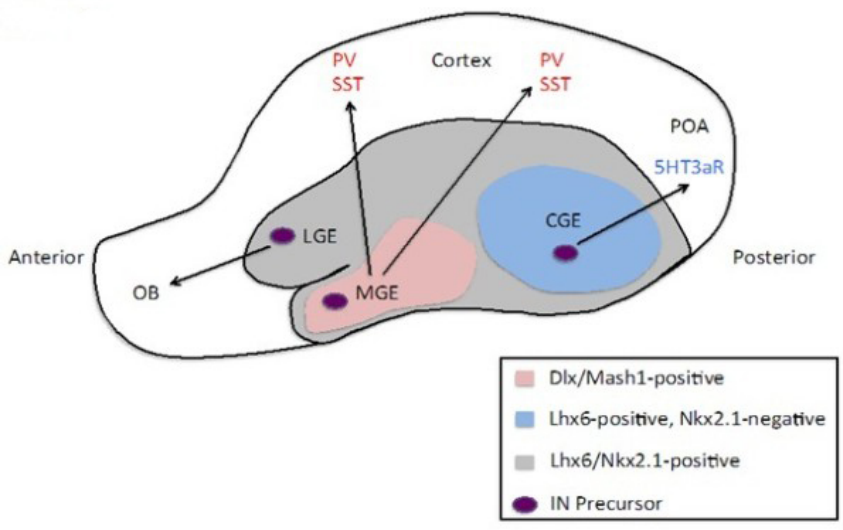

Figure 5 Origin of PV interneurons

A sagittal view of the telencephalon of the embryonic telencephalon at approximately embryonic day 13.5 (E13.5) showing the major origins of GABAergic cortical interneurons. The MGE represents the Lhx6- and Nkx2.1- positive area.

1.2.4 Feed-forward inhibition

Some interneurons are innervated by afferent inputs from remote principal cells. They usually have faster spike latencies than the principal cell. Therefore, the inhibition mediated by those interneurons is defined as feed-forward inhibition (FFI). On contrast to feed-back inhibition, which is mainly driven by the activity of local principal cells, feed-forward inhibition reflect the strength of afferent inputs, exert direct control on the spike timing and perform lateral inhibition to silent part of the principal cell population. Therefore, feed-forward inhibition is thought to be important in signal-noise ratio in neural computation. The kinetics, synaptic plasticity and dendritic location of FFI event have a great influence on the spatial and temporal range of FFI. PV cells mediate fast FFI and selectively innervate the peri-somatic region of principal cells. Meanwhile, PV cells usually fire within a short time window and the I-E synapses on principal cells are usually depressed with multiple spikes. Therefore, PV cells have a strong and precise timing control at the initiation of activity of principal cells but have a relatively weak effect on spike frequencies, which could be regulated by feedback inhibition.

1.3 Experience dependent neural plasticity

1.3.1 Basis of Experience dependent neural plasticity

Neural plasticity refers to the ability of neural circuits to change according to the past experience or activity. The change could be short term or long term, and range from synaptic weight to the tissue structure. Here we mainly discuss how training experiences such as fear conditioning and environmental enrichment change the related neural circuits.

Fear conditioning is a behavioural paradigm that the subjects learn to predict aversive events. In fear conditioning, an aversive stimulus (e.g. an electrical shock) is repetitively associated with a certain neutral context or stimulus (e.g., a room or a tone), which resulted in the expression of

fear responses to a stimulus or context that is originally neutral. Fear conditioning is thought to depend on amygdala, which is important in fear and other emotions. Damage or inhibition of the amygdala can prevent both the learning and expression of fear. Contextual fear conditioning also involve the hippocampus. The neural plasticity underlying fear conditioning can be detected in synapse and circuit level.

Environmental enrichment (EE), on the other hand, stimulates the brain in multiple aspects by its physical and social surroundings. The diversity and continuous stimulation from the environment is beneficial to neural development. Associated with the extra synapses, there is also increased synapse activity and increased size and number of glial energy-support cells. Environmental enrichment could also boost vasculation of capillary, providing the neurons and glial cells with extra energy, which could explain why EE could enhance the cognitive function in many ways.

1.3.2 Plasticity of intrinsic neuronal excitability

Modification in intrinsic excitability may contribute to the formation of functional neuronal assemblies and specific memory traces. The first evidence for intrinsic plasticity came from the pioneering work of Alkon who showed that phototaxic learning in the marine mollusk Hermissenda crassicornis involved the reduction of voltage-gated A-type and Ca^{2+}-activated K currents in a photoreceptor (Alkon, 1984). Several forms of learning (sensitization and operant conditioning) in Aplysia were also found to be associated with persistent changed in neuronal excitability (Scholz and Byrne, 1987; Mozzachiodi and Byrne, 2010).

In central cortical neurons, due to the interaction between synaptic and intrinsic voltage-gated conductance, excitatory postsynaptic potentials (EPSPs) measured at the axon hillock were either amplified or attenuated compared to the amplitude measured at synaptic site (Spruston, 2008). Thus regulation of ion channel activity represents a powerful means to control the dendritic integration of principal neurons. For instance, tetanic stimulation of glutamatergic synaptic inputs not only induces LTP in hippocampal neurons but also persistently facilitates the generation of a postsynaptic action potential by the EPSP (Bliss et al., 1973; Andersen et al., 1980), a phenomenon known as EPSP-Spike potentiation. Especially, this form of plasticity is also

bidirectional. Induction of LTD with low frequency stimulation of the Schaeffer collateral-CA1 neuron input is associated with a long-lasting decrease in EPSP- Spike coupling (Daoudal et al., 2002). Moreover, evidences from in vivo studies showed that repetitive postsynaptic bursting by current injection produced a long-lasting (> 30 min) increase in intrinsic neuronal excitability of the great majority of neurons (Paz et al. 2009).

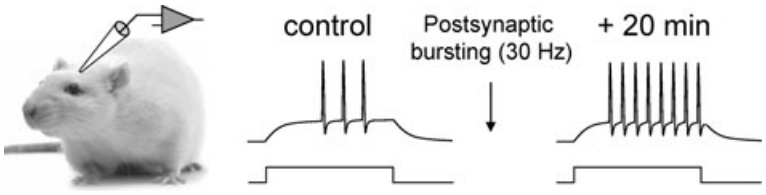

Fig 6. In vivo intrinsic neuronal plasticity

Repeated bursting of layer 5 pyramidal neurons induces long-term potentiation of intrinsic excitability in rat cortical motor neurons *in vivo.*

1.3.3 Neural plasticity of inhibitory transmission

During critical periods of early life, there is a time window of enhanced plasticity that is attributed to the differentiating parvalbumin (PV)-interneuron network. Therefore, recent experience may modulate learning by targeting the differentiation state of PV neurons. Works from our lab show that learning of maze navigation induces a low-PV-network configuration in hippocampus. Memory and structural synaptic plasticity are enhanced throughout training, which followed by a shift to a high-PV-network configuration in hippocampus after learning completion (Donato et al., 2013).

PV^+ basket cells in hippocampal CA1 have recently been shown to be induced a long term increase in intrinsic excitability with a brief and high frequency stimulation of afferent excitatory inputs (Campanac et al., 2013). The increase in excitability of PV interneurons results from activation of mGluR5 and the down-regulation of D-type potassium, which leads to an increased feed-forward inhibition mediated by PV cells. Excitatory synapses onto interneurons can express long-term potentiation or depression via activation of calcium-permeable glutamate receptors (Kullmann and Lamsa, 2007). The excitatory inputs onto hippocampal interneurons have been

shown to express multiple forms of long-term, activity-dependent plasticity in recent studies (Kullmann & Lamsa, 2007; Pelletier & Lacaille, 2008). Hebbian LTP that is NMDA receptor dependent has been observed at FF inputs onto some st. radiatum interneurons (Lamsa et al. 2005). It contributes to the temporal fidelity of synaptic integration in principal cells. Another form of LTP that depends on calcium-permeable (CP-) AMPARs was reported in several interneuron subtypes (Lamsa et al. 2007; Szabo et al. 2012). Different from NMDAR-dependent LTP, coincident postsynaptic hyperpolarization (not activation) and presynaptic activation are required for such form of plasticity and was thus named anti-Hebbian (Lamsa et al. 2007).

PV cells establish predominantly perisomatic synapses onto pyramidal neurons. Therefore, they are well suited for controlling the discharge of their postsynaptic targets (Pouille & Scanziani, 2001). Moreover, the axonal divergence of PV cells allows them to synchronize the firing of large neuronal ensembles effectively (Cobb et al. 1995). The efficacy of excitatory inputs onto PV cells have great impact on the spatial and temporal range of PV cells' control over principal cell activity (Racz et al. 2009; Korotkova et al. 2010). Activity-dependent synaptic plasticity at these excitatory synapses onto PV cells may be required to maintain the precision in timing of the operation of PV cells.

1.4 Hypotheses

1.4.1 Modulation of inhibitory transmission by experience

With multiple reports of PV interneuron plasticity in the hippocampus, we think it is important to determine the neurophysiological outcome of PV interneuron plasticity as a result of behavioural learning and to examine how the plasticity of PV interneurons contributes to the hippocampus' ability of mediating the processing and storage of information.

Recent studies about PV interneuron network state of adult animals show the latent capacity of a microcircuit module to regulate plasticity and learning (Donato et al., 2013; Ruediger et al., 2011). As such, a neurophysiological zoom-in and demonstration of the basic elements in inhibitory microcircuit could provide a better understanding for this regulation upon behavioural learning. Considering the importance of intrinsic neuronal excitability in learning, first we would like to have direct evidences for the plastic change in PV interneuron intrinsic excitability upon

experience. Furthermore, by measuring Schaeffer collateral-mediated di-synaptic feed-forward inhibitory transmission in CA1, we hypothesized that the plasticity in the strength of feed-forward inhibitory transmission, like the more commonly studied plasticity in glutamatergic transmission, would underlie the learning in adult microcircuits. Taken together, we would like to build the physiological link between PV interneuron network state and behavioural learning. Hence, we performed whole cell patch clamp recordings in acute slices from adult mice on fast-spiking PV cells and tried to examine intrinsic neuronal excitability of these cells. In addition, CA1 averaged group of principal cell was subsequently recorded to study feed-forward inhibition in the hippocampal circuit.

1.4.2 Functional heterogeneity in hippocampal CA1 principal cells

Evidences are accumulating that there are functional heterogeneity in hippocampal CA1 principal cells. Previous works in the lab show that CA1 pyramidal cells highlighted by membrane-bound GFP in the same mouse line could be considered as distinguishable subpopulations. Considering the importance of information about intrinsic neuronal excitability when interpreting the functional significance of certain genetic, synaptic and connection specificity of different subpopulations, we hypothesized intrinsic neuronal excitability would be a potential physiological candidate for shaping functional distinct circuits. Moreover, the inhibitory circuit involving these subpopulations of principal cells should also be shaped differentially by experience. Hence, we performed recordings in acute slices from juvenile and adult mice on Lsi1 and Lsi2 subpopulation of principal cell to examine that intrinsic neuronal excitability between these two subpopulations of cells. Feed-forward inhibition onto Lsi1 subpopulation of principal cells was subsequently studied to better understand how inhibitory circuits were shaped upon experience.

2

Methods and Materials

Methods and Materials

Animals

Mouse line PV–Cre, B6; 129P2-Pvalb$^{tm1(cre)Arbr}$ were crossed with mouse line Rosa-CAG-STOP-tdTomato (kindly provided by Dr. Sylvia Arber) to produce PV-RFP mice.

Thy1-mGFP (Lsi1 or Lsi2 sparse labelling line) mice were used for the study of subpopulations of principal cells. Mice expressing GFP constructs specifically in adult neurons were generated using the mouse Thy1.2 expression cassette. mGFP consisted of the first 40 amino acids of the protein MARCKS, a plasmalemmal targeting sequence46, fused to enhance GFP (eGFP).

All mice were maintained with a 12 h light/dark cycle. Food and water was provided ad libitum. All the procedures conformed to the Institutional guidelines and were approved by the Veterinary Department of Cantonal Basel-Stadt.

Brain slice preparation

400 µm hippocampal slices were prepared from young adult P60-90 days old (except where noted) mice. Mice were anaesthetized with isofluoran, and perfused through the ascending aorta with chilled modified artificial cerebrospinal fluid (ACSF). Modified cutting ACSF (Ting et al., 2014) was composed of 92 mM NMDG, 92 mM HCl , 2.5 mM KCl, 1.2 mM NaH_2PO4, 30 mM NaHCO3, 20mM HEPES, 25 mM glucose, 10 mM MgSO4, 0.5 mM $CaCl_2$, 5 mM sodium ascorbate, 3 mM sodium pyruvate and 2mM thoiurea and saturated with 95% O_2/5% CO_2 (pH 7.3-7.4, osmolarity ~310 mOsm). After cardiac perfusion, the brain was quickly removed, and hemispheres were separated and placed into the chilled solution for another 3 minutes. One hemisphere was placed on a dish, with the sagittal plane down and the temporal surface up. The hemisphere was trimmed according to protocol modified from P. Jonas lab and was mounted on section tube, and then embedded with low melting temperature agrose. 400 µm slices were cut with a Compresstome™ Microtome VF-200 (Precisionary Instruments). Slices initially recovered in cutting ACSF for 10 minutes and then were transferred to recovering ACSF for further incubation about 1 hr at room temperature. Recovering ACSF is composed of 92 mM NaCl, 2.5

mM KCl, 1.2 mM NaH_2PO_4, 30 mM $NaHCO_3$, 20mM HEPES, 25 mM glucose, 2 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM sodium ascorbate, 3 mM sodium pyruvate and 2mM thoiurea and saturated with 95% O_2/5% CO_2 (pH 7.4, osmolarity ~310 mOsm). Subsequent recording within 6 hrs showed no difference in electrophysiological properties among neurons.

Recording

Recordings were performed with physiological ACSF containing 130 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 30 mM $NaHCO_3$, 10 mM glucose, 2 mM $MgSO_4$, 2 mM $CaCl_2$ in a recording chamber at room temperature at a perfusion rate of 1-2 mL/min. Neurons were visually identified with infrared CCD camera (HAMAMATSU C2400) using an upright microscope (Zeiss Axioskop) equipped with a 40X objective. Parvalbumin interneurons neurons were identified by fluorescent RFP while Lsi1 pyramidal neurons were identified by fluorescent GFP.

Whole-cell recording pipettes were pulled from borosilicate glass tubing (Science products, GB150F-8P) with a NARISHGE vertical puller to resistances of 3-5 MOhms. When studying the intrinsic properties of hippocampal cells, pipettes were filled with normal intracellular solution (K-gluconate based) consisting of 130 mM K gluconate, 6 mM KCl, 10 mM HEPES, 0.2 mM EGTA, 4 mM Mg ATP, 0.3 mM Na2GTP, 10 mM phosphocreatine and 0.1% biocytin (pH 7.3, osmolarity 290 mOsm); When exploring the feed-forward excitation and inhibition onto CA1 pyramidal cells, Cs based intracellular solution was used: 140 mM Cs-methanesulphonate, 6 mM KCl, 10 mM HEPES, 0.1 mM EGTA, 4 mM Mg ATP, 0.3 mM Na2GTP, 8 mM phosphocreatine, 0.5mM QX-314 and 5mM TEA (pH 7.3, osmolarity 290 mOsm).

All membrane potential values in the text and figures were uncorrected for the liquid junction potential. Signals were amplified using an Axon Instruments Multiclamp 700b and digitized using an Axon Instruments Digidata 1440A with a sampling rate of 10 kHz. Data were acquired and analyzed with Clampex 10.0, Clampfit 10.0 (Molecular Devices). Data are the mean ± SEM. p values are calculated by paired t-test.

Whole cell patch-clamp recordings were excluded if the access resistance was higher than 40 MΩ and changed more than 20% during the recordings.

Extracellular stimulation

Trains of extracellular stimuli were applied using a concentric bipolar stimulating electrode (FHC,

Bowdoinham, ME) placed in Schaeffer-collateral pathway. The distance between stimulating electrode and CA1 pyramidal layer is fixed (50 μm) and the tangential distance to recorded cell is controlled as well (150-200 μm). The stimulus was generated by an AMPI ISO-Flex stimulus isolator triggered by an AMPI Master 8 stimulator, and was 1ms in duration with intensities ranging from 2.5V to 12V. Stimulus trains consisted of 10 pulses, presented at 0.1 Hz.

Pharmacological agents

CNQX (Sigma) was used to block glutamatergic transmission in all recordings of isolated IPSCs. Picrotoxin (Tocris), and CGP54626 (Tocris) was used to block GABAergic transmission in all recordings of isolated EPSPs. DAMGO (Sigma) was used to block Parvalbumin interneuron mediated inhibitory transmission, while WIN55, 212 (Sigma) was used to block CCK interneuron mediated inhibitory transmission. Biocytin (Vector Laboratories Inc.) was mixed in intracellular solution to trace recorded cell by staining afterward.

Biocytin staining and immunostaining

Target cells were labelled by biocytin via recording pipettes. After completing the entire electrophysiological recording in whole cell configuration, the electrode was slowly retracted. Brain slices were then incubated for 5 min more in recording chamber, and subsequently stored in 4% paraformaldehyde for 1hr at room temperature and transferred in PBS for temporary storage. Later slices were incubated with streptavidin 568 conjugates (Molecular Probes, 1:500) to visualized recorded neurons. The inclusion of Parvalbumin antibody and WFA lectin further characterized the identity of recorded neurons. WFA is a lectin that specifically binds to chondroitin-sulfate side chains that are mainly present on chondroitin-sulfate proteoglycans (CSPGs) in the dense perineuronal nets (PNNs) surrounding parvalbumin-positive interneurons. Goat anti-PV (Swant biotechnologies, 1:5000) and Alexa 647 Conjugate (Molecular Probes, 1:500) were used to label PV cell and later quantify PV intensity. WFA lectin conjungated GFP (Vector Labs, 1:500) was used to visualize perineuronal nets.

Electrophysiology data analysis

By whole cell recording we got the intrinsic properties of targeted cells. The firing pattern of interneurons and pyramidal cells was obtained immediately after achieving whole-cell

configuration via steps of current injections. Square pulses of current (duration 1000 ms) were delivered at 0.1 Hz. The firing frequency was determined by the steady-state spike frequency within initial 50 ms after the onset first spike. For PV neuron study, in response to 1s depolarization current step, cells with maximal firing frequency higher than 50 Hz is characterized as fast-spiking interneurons. Rheobase measurement (applying minimal amount of current to reach the firing threshold) was used for acquiring other firing properties. Action potential (AP) thresholds were determined by finding the average voltage at which the second derivative of the trace was maximal, within 5 ms preceding the action potential peak. The AP amplitude was determined from AP threshold to peak, and the half-width as the width of the depolarizing spike at 50% of spike amplitude. Fast AHP (fAHP) was defined as the difference between the peak of the rapid hyperpolarizing voltage following a single AP and the threshold of the AP.

Voltage clamp was used for feed-forward inhibition measurement. Postsynaptic synaptic currents were evoked by Schaeffer collateral pathway stimulation. The minimal voltage used to elicit excitatory postsynaptic currents was first acquired and to be used as normalization parameter. With 2 times of minimal stimulation intensity, excitatory PSCs (EPSCs) were measured at holding potential -70mV while inhibitory PSCs (IPSCs) were measured at 0mV. Subsequent values were used to compare among different experimental configurations.

Imaging analysis

PV intensity was analysed with protocols described as before (Donato et al., 2013). PV interneurons somas were isolated and PV intensity was quantified automatically via Imaris (Bit plane). Averaged PV intensity was used for PV interneuron categorization and comparison among treatments.

Quantification of perineuronal nets around PV cells was implemented by image analysis of wisteria floribunda agglutinin (WFA) fluorescence intensity. Image segmentation by Matlab (MathWorks) produces a shell like structure around PV cell. Since we found that the total fluorescence intensity within 2 micrometers distance from the cell body can reflect the difference in WFA intensity among individual neurons, we employed this parameter as WFA intensity indicator for further comparison and analysis.

Behaviour

Contextual Fear-Conditioning. 8 weeks old mice were separated 1d before the acquisition. On the training day, the acquisition box was first cleaned with 70% ethanol and was smeared with 1% acetic acid before the acquisition. Once a mouse was put into the acquisition box, it was allowed to explore freely the box for 2.5 min, and then 5 foot-shocks at an intensity of 0.8 mA were sequentially delivered (with 1s duration, 30s inter trial interval). 24 hrs later, the mice were sacrificed for electrophysiological recording.

Environmental enrichment. Female mice around postnatal 5 weeks were housed in groups of five to six in large cages equipped with toys, two running wheels per cage, and hiding spaces. 3 weeks later, mice were sacrificed for electrophysiological recording. Control mice around 8 weeks were female (three to six), which were housed individually in small standard cages without special equipment.

3

Result I

Learning modulation of inhibitory transmission in hippocampal CA1 area of adult mice

3.1 Summary

One of the most intriguing features of the neural circuit is that previous experience or activity could modify neural circuit and leave their structural and functional impacts, with a duration that depends on the form of a particular experience. Previous work from our lab showed that Pavlovian contextual fear conditioning (cFC) and environmental enrichment (EE) differentially modulate parvalbumin (PV) interneuron network. Contextual fear conditioning shifts PV interneuron network towards high PV configuration (larger fraction of high PV-expressing and GAD67-expressing PV cells), while environmental enrichment drives PV interneuron network to low PV state (larger fraction of low PV-expressing and GAD67-expressing PV cells, Donato et al., 2013). However, the neurophysiological consequence of such modulation is not clear.

The aim of my thesis study is to reveal the physiological relevance of experience-induced changes in PV interneuron network, and to investigate how these changes affect information processing of excitatory microcircuits.

Methodologically, acute brain slices are obtained from adult mice for electrophysiological recordings. Parvalbumin interneurons in hippocampal CA1 are visualized through genetically labelling by green fluorescent protein (GFP). By whole-cell patch clamp recording, we showed that fast-spiking PV interneurons were not homogeneous. They exhibited wide range of firing frequencies, distinct firing patterns, and diverse intrinsic membrane properties. Interestingly we found cFC could modulate these electrophysiological properties of PV cells. Contextual fear conditioning homogenized the firing behavior of PV cells. More PV cells fired with increased firing rates and showed no spike adaptation (adaptation ratio near 1.0). After cFC, there was a better correlation between firing frequency and adaptation ratio. On the contrary, environmental enrichment diversified the firing behavior of PV cells with a wider spectrum of firing frequency distribution and showed more pronounced spike adaptation. This resulted in diminished correlation between firing rate and adaptation ratio. Moreover, results from subsequent biocytin and peri-neuronal nets staining indicated that cells with higher firing frequency were usually surrounded by higher amount of peri-neuronal nets. Considering the linear correlation between PV expression level and peri-neuronal nets, cells fire at high

frequencies tend to be high PV cells; thus the shift towards higher PV expression after cFC is physiologically linked to a higher firing frequencies in PV cells.

We further examined whether such intrinsic plasticity of PV cells could modify hippocampal CA1 inhibitory circuits. We expected enhanced inhibitory transmission in CA1 area due to increased firing rates of PV cells after cFC. Indeed, we observed that total inhibition and feed-forward inhibition provided by PV interneurons onto principal cells increased significantly as measured by Schaeffer collateral stimulation in acute slices of adult mice. As expected based on results above, EE diversified its modulation on inhibitory transmission. Total inhibition exhibited two distinct subgroups while feed-forward inhibition provided by PV interneurons onto principal cells decreased significantly after EE.

In conclusion, by combining neurophysiology with behaviour and histology, we show that cFC can modify fast-spiking PV^+ interneurons by modulating both intrinsic properties and inhibitory transmission in feed-forward connections in adult mice. Further research is required to study the causal link between the intrinsic excitability alterations and PV cells at different differentiation state (categorized as high PV and low PV cells) as well as how this plasticity of inhibitory circuit supports learning completion and memory precision.

3.2 Introduction

Learning modifies behaviour through experience and modulates information flow at the level of neuronal circuits. Activity-dependent plasticity and structural modifications are thought to be the cellular mechanisms underlying learning at the level of defined brain microcircuits where excitatory and inhibitory neurons interact to execute information processing. The distinct wiring pattern in defined brain microcircuits and physiological properties of individual neurons enable the specific brain network to carry out the computation. Both synaptic plasticity and intrinsic plasticity contribute to the plastic brain microcircuits for learning and memory.

In past decades, activity-dependent plasticity of excitatory synaptic circuits has been studied extensively. In the aspect of synaptic strength, early studies on simple behaviours of eye-blink response in rabbits (Thompson et al. 1983) and the gill-withdrawal reflex in Aplysia (Kandel and Tauc 1963) provided direct evidence that learning can modify synaptic connections between neurons. These anatomical modifications serve as elementary components of memory storage (Carew et al., 1981; Bailey and Kandel 1993). Several other studies on amygdala, a brain structure essential for both innate and learned fear, strongly linked learning and memory to synaptic long-term potentiation (Davis et al. 1994; LeDoux 1995, 1996). In the aspect of structural synaptic plasticity, learning new skills involves formation of new synapses (Hofer et al., 2009; Xu et al., 2009; Yang et al., 2009). In the aspect of intrinsic plasticity, activity-dependent persistent plasticity of intrinsic neuronal excitability after LTP has been reported in CA1 hippocampal principal cells (Campanac et al., 2008; Campanac and Debanne, 2008; Daoudal et al., 2002; Wang et al., 2003).

Excitation and inhibition execute functions side by side in cortical circuits (Isaacson and Scanziani, 2011). This excitatory-inhibitory balance is thought to maintain activity within physiological bounds and shape cortical activity in space and time scale. Moreover, functions of interneuron have been shown to be critical for various brain waves such as theta oscillations in the hippocampus, which is essential for successful learning (Buzsaki, 2002; Klausberger and Somogyi, 2008).

Activity-dependent plasticity of inhibitory circuits is achieved by enhancing excitatory synaptic drive to inhibitory interneurons (Alle et al., 2001; Kullmann and Lamsa, 2007; Kullmann et al., 2012; Lamsa et al., 2007; Maccaferri et al., 1998; Pelletier and Lacaille, 2008). For instance, both feed-forward and feedback

interneurons in the CA1 region of the hippocampus express long-term synaptic potentiation (Lamsa et al., 2005; Lamsa et al., 2007). Moreover, high frequency stimulation of SC input can lead to a long-term increase in excitability of PV^+ basket cells in CA1 area (Campanac et al., 2013). However, there is no direct evidence of behavioural learning-induced inhibitory synaptic plasticity and intrinsic excitability alterations.

Recent studies showed that excitatory connectivity onto PV interneurons in the hippocampus is plastic and can be modified by contextual fear conditioning (cFC). Learning completion could produce an increase in the number of excitatory filopodial synapses onto fast-spiking interneurons in hippocampal CA3, which is necessary for memory precision upon learning (Ruediger et al., 2011). Following studies showed that the change in excitatory input shifts parvalbumin and GAD-67 expression to higher levels (Donato et al., 2013). What is the physiological impact of learning-induced changes in connectivity onto PV^+ interneurons, and how could this impact information processing of brain microcircuits?

Here we systematically examined functional properties of PV cells, and showed contextual fear conditioning modified basic electrophysiological properties of fast-spiking PV^+ cells. In addition, feed-forward inhibition is mostly enhanced by fear conditioning that results in higher inhibitory output measured with Schaeffer collateral stimulation.

3.3 Result

1. Functional modulation of hippocampal fast-spiking PV^+ cells by contextual fear conditioning.

Previous studies from our lab have described the structural plasticity in inhibitory neural circuit in hippocampus (Ruediger et al., 2011; Donato et al., 2013). It is very intriguing to understand whether learning experience also affects the function of hippocampal PV-network, and whether different learning paradigms have distinct effects. We tested this idea by examining the most basic but important neurophysiological feature, the excitability, of PV cells in hippocampal CA1 area.

By using contextual fear conditioning and environmental enrichment paradigm described before (Donato et al., 2013), we trained 5-8 weeks old mice (PV-Cre :: Rosa-CAG-STOP-tdTomato), in which PV neurons were genetically labelled with a reporter protein tdTomato for visualization. The change of PV expression level upon two different learning paradigms was consistent with previous results (Donato et al, 2013), further confirmed learning-specific plasticity of hippocampal PV network. The hippocampal brain slices were prepared acutely from both caged control and trained animal for whole cell recordings and immunostaining of PV and binding of WFA lectin. By whole cell recording, steps of current were injected into PV cells to test their excitability. Initial firing frequency was calculated by measuring spike frequency in response to intracellular current injection at 300 pA (Figure.1B).

Our result showed that fast-spiking PV cells in hippocampal CA1 region are heterogeneous (Figure. 1C). They exhibited wide spectrum of firing frequency ranging from 50 Hz to 140 Hz. Furthermore, similar as the structural modulation, different learning paradigms have distinct impact on the excitability of these PV cells. The firing frequency of fast-spiking PV cells increased significantly after cFC (Figure 1C. Ctrl: 89.89 ± 5.026 Hz, n=18; cFC: 103.5 ± 3.929 Hz, n=20; $p = 0.038$), suggesting PV cells become more sensitive to the afferent stimulus and exert a stronger inhibitory control on hippocampal principal cells; On the other hand, EE did not reduce firing rates as expected, the averaged value of firing frequency showed no significant change (Ctrl: 89.89 ± 5.026, n=18; EE: 91.65 ± 7.364, n=16; p=0.77). However, the individual values exhibit a large variability and there are subgroups: one group of cells with higher firing frequency and another group with lower firing frequency. It suggests the EE experience may not have

homogeneous influence on the spike output of PV cells; the modulation is allocated to different subgroups of PV cells.

These results demonstrate that the excitability of PV interneurons can be modulated by contextual fear conditioning and environmental enrichment. This neurophysiological change could serve as a cellular mechanism for behavioural learning in hippocampus.

Figure 1

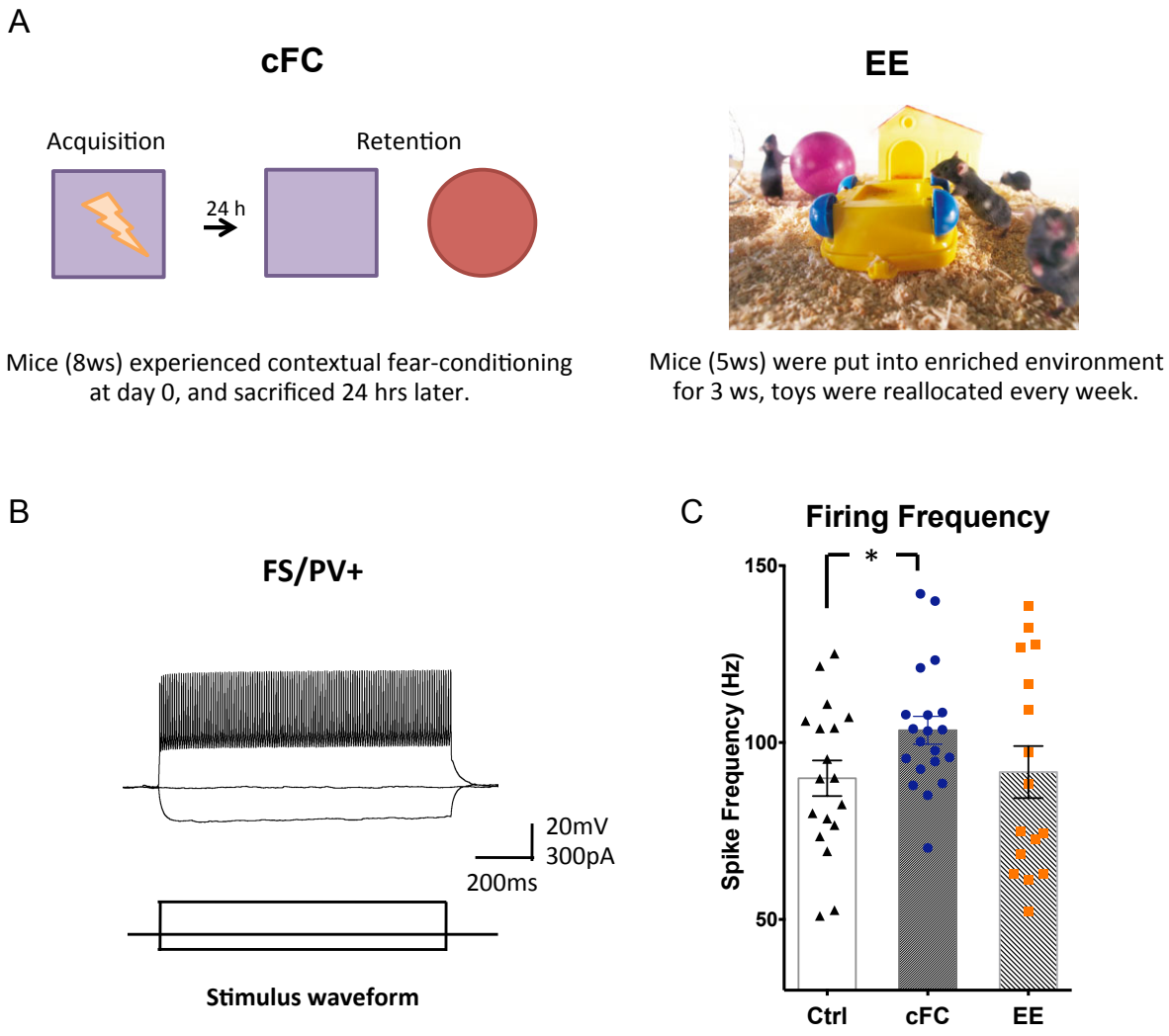

Figure 1: Experience modulate firing rates of fast-spiking hippocampal CA1 parvalbumin-expressing cells in the adult mice

A. Eight or five weeks old mice were trained with Pavlovian contextual fear conditioning (cFC) or environmental enrichment (EE) behavioral paradigms. Subsequently, hippocampal acute brain slices were prepared 24 hours after cFC training or 3 weeks after EE training.

B. Typical firing pattern of fast-spiking PV^+ cells. The lower panel was stimulus waveform; the upper panel showed the hyperpolarization and depolarization of PV cells in response to 1 s intracellular current injections at -100 pA, 0 pA and 300 pA.

C. Scatter plot of spike frequency in PV fast-spiking cells upon different learning experiences. Initial firing frequency was calculated by spikes in response to intracellular current injection at 300 pA. Unpaired t-test was made between experimental and control groups. Ctrl vs. cFC exhibited significance with $P = 0.0384$. The asterisk represents statistical significance ($p < 0.05$); error bars indicate SEM.

2. Distinct firing patterns of PV-expressing fast-spiking interneurons and the modulation by experience

Our result showed that all the PV-expressing fast-spiking interneurons fire at high frequency during the initial phase of current injection (300 pA, 1s), but their response to the late phase of current injection are quite diverse: some showed no significant adaption, some fired with frequency adaptation and some stopped firing at all. According to their spike frequency adaption, we classified them into three groups: Non-accommodating (NA) with no spike frequency adaptation, accommodating (A) and strong accommodating (SA) (Figure. 2A). Spike frequency adaptation was then calculated and quantified as adaptation ratio: the ratio of the first inter-spike interval (1st ISI) to last (Last ISI). The NA type is the classical firing pattern for PV fast-spiking interneurons with higher adaption ratio larger than 0.5 and has the highest firing frequency (Figure. 2C).

We further characterized the fraction of these three types of PV cells in control and experimental groups. Interestingly, experience can dramatically change the ratio of these three types (Figure. 2B). Contextual fear conditioning group has larger fraction of NA type (73%), while in the environmental enrichment group majority of cells have adaptation ratio between 0.3-0.5, which belong to A type (60%). The A type cell fire in a similar way to the NA type upon brief stimulus, but their response gradually decrease when the stimulus was prolonged.

To determine the functional indication for the modulation of firing pattern, we plotted adaptation ratio with firing frequency in pooled PV cells. A significant correlation was found between these 2 parameters (Pearson correlation $r = 0.3639$, $P = 0.0074$; Figure. 2C), suggesting cells with higher value of adaptation ratio tend to fire higher firing rate. However, the correlation in each experimental configuration behaved differently. The cFC group exhibited a better correlation (Pearson correlation $r = 0.5383$, $P = 0.0118$), while EE group lost this correlation completely ($r = 0.08228$, $P = 0.7797$) and Ctrl group did not show a significance ($r = 0.3811$, $P = 0.1187$). These results suggest contextual fear conditioning homogenize PV cells and drive more cells to NA type with increased firing rates, while EE diversified the firing behavior of PV cells with similar adaptation ratio and diminished the correlation between firing frequency and adaptation ratio.

Moreover, the delay onset of first spike in response to rheobase current injection was also examined. Early onset was defined as onset time less than 30 ms. Our analysis showed experience did not change the incidence of early and late onset cells (Figure. 3B).

To give a complete description of intrinsic properties of PV cells before and after experience, and to explain the physiological mechanism of modulation on PV cell firing pattern by cFC and EE, we further characterized the other six indices of intrinsic properties that underlie the firing pattern: input resistance (Rm), spike amplitude (Amp), spike half-width (t1/2), resting membrane potential (Vm), After-hyperpolarization potential (AHP) and spike threshold (Vt). Compared with caged control, contextual fear conditioning did not significantly change these intrinsic properties; however the parameters of Rm, Amp, t1/2, and Vm showed similar tendency to support cells firing at higher frequency with less adaptation (Figure. 3A). Decreased Rm leads to faster electrical charging during spike generation and higher firing frequencies. Decreased spike half-width and Amp result in less sodium channel inactivation which is consistent with less adaption after cFC.

Figure 2

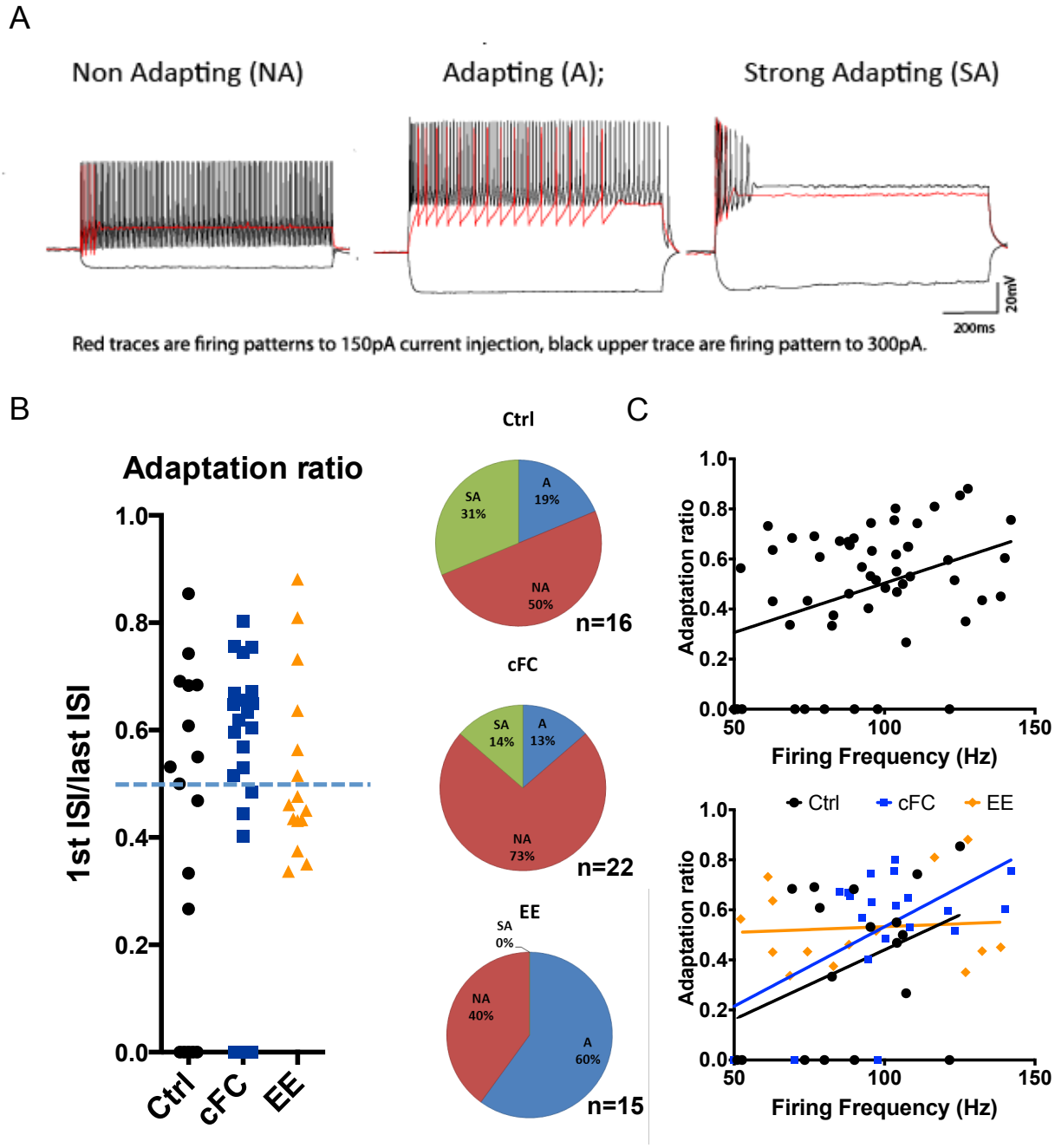

Figure 2. Three typical firing patterns of fast-spiking PV cells and the modulation by experience.

A. Three typical firing patterns of PV cells in CA1 hippocampus: non-adapting (NA), adapting (A) and strong adapting (SA). The majority of PV cells were 'NA' in cFC while the 'A' type increased from 19% to 60% after EE.

B. Spike adaptation was quantified via adaptation ratio of 1st ISI/ last ISI. Experience modulated the distribution of adaptation ratios. Cells with the ratio >0.5 is defined as NA cells; <0.5 as A cells; SA cells were easily be identified since they adapted dramatically thus could not fire until the end of 1 s current stimulation, in which their adaptation ratio is 0.

C. Correlation between adaptation ratio and firing frequency. Upper panel is the correlation derived from pooled cells (Pearson correlation r=0.3639, P=0.0074). Lower panel is the linear fit for individual experimental group. The correlation is significant in cFC group (cFC: Pearson correlation r = 0.3639, p =0.0074; Ctrl: Pearson correlation r = 0.3811, p = 0.1187; EE: Pearson correlation r = 0.08228, p = 0.7797)

Figure 3

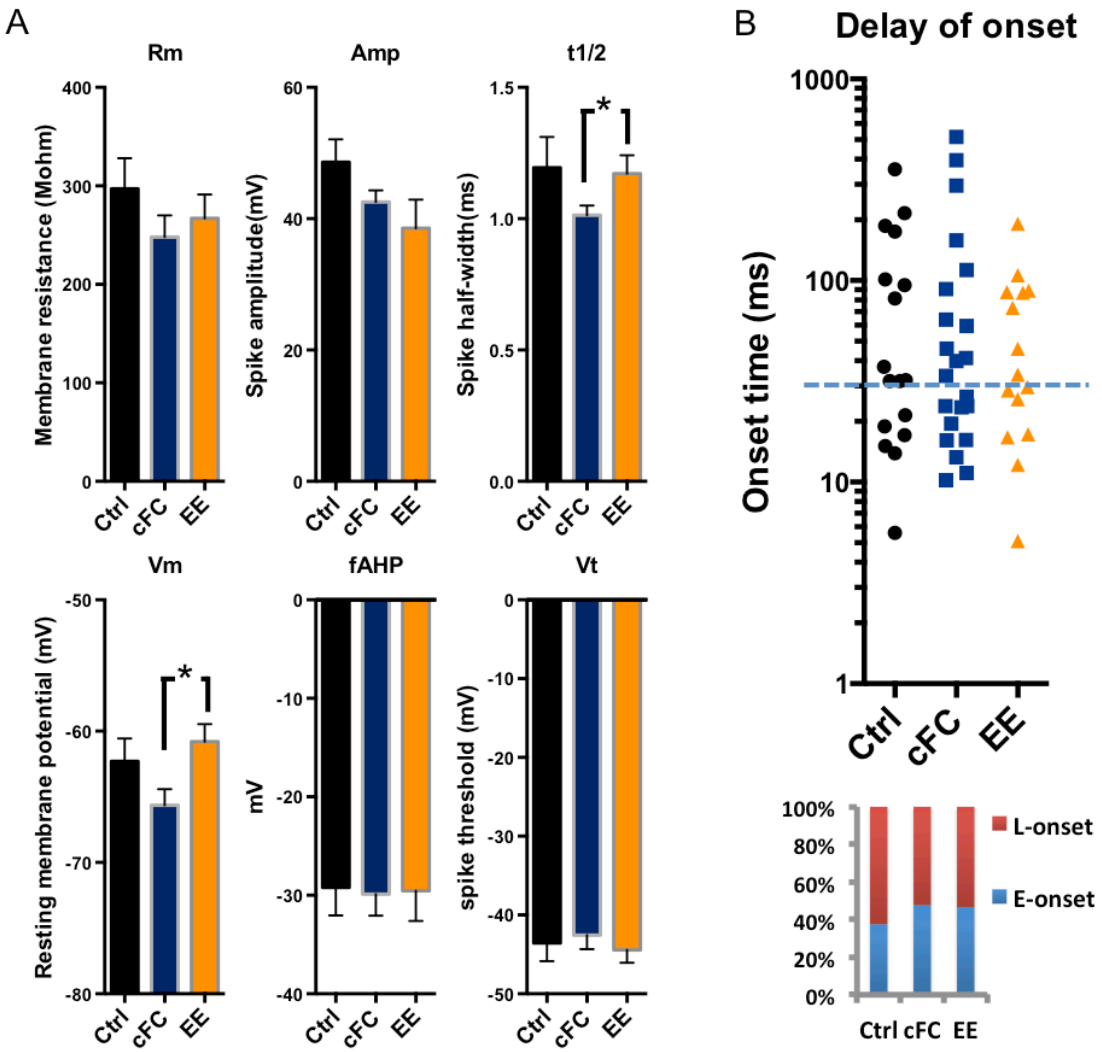

Figure 3 Intrinsic properties of PV cells and their modulation by experience

A. Intrinsic properties of PV cells upon 300 pA current injection for 1 s. Input resistance (Rm), spike amplitude (Amp), spike half-width (t1/2), resting membrane potential (Vm), after-hyperpolarization potential (AHP), and spike threshold (Vt) were measured from PV cells in control, cFC and EE groups.

B. The behavioural training reshaped the fraction of different firing pattern distribution but had no effect on the onset time of single spike in rheobase stimulation.

3. Modulation of feed-forward inhibition onto CA1 pyramidal cells by experience.

It was shown that the firing frequency of fast-spiking PV cells and other electrophysiological intrinsic properties were altered after cFC (Figure. 1C). Given the fact that recent experience regulates excitatory and inhibitory inputs onto PV interneurons and high PV and low PV interneurons would be differentially recruited (Donato et al., 2013), we further examined the functional inhibitory output after such alterations in PV interneurons.

To investigate the impact of PV network shift on the hippocampal CA1 circuit, we measured feed-forward inhibition in dorsal CA1 area of acute hippocampal slice from adult mice. Feed-forward inhibition (FFI) plays important roles in setting magnitude and duration of neural activity, controlling spike timing and synaptic plasticity, regulating signal-noise ratio in the neural information processing (Pouille and Scanziani, 2001). Furthermore, fast-spiking PV cells are the main source of perisomatic FFI.

A stimulus electrode was placed at stratum radiatum in CA1 to stimulate the Schaeffer collaterals fibres to provide feed-forward transmission. Whole cell recording was made on CA1 pyramidal cells to record direct excitation, di-synaptic feed-forward inhibition and tri-synaptic feedback inhibition (Figure. 4A and 4B). Total evoked IPSCs were obtained by holding the membrane potential at 0 mV. Sequential administration of u-opioid receptor agonist DAMGO (100 nM) and CB1 receptor agonist WIN55-212 (1 uM) isolated PV- and CCK-sensitive components respectively. By subtracting the recorded IPSC before and after DAMGO/WIN55-212 application, we can calculate the pure PV-mediated (red) and CCK-mediated (blue) IPSC components (Figure. 4C).

Comparing these IPSCs measured in control condition with those measured in preparations from contextual fear conditioning and environmental enrichment trained animals, we found that after cFC the total feed-forward inhibition (total IPSC) in pyramidal cell increased (Control: 567.3 ± 50.20pA, n=13; cFC: 759.0 ± 63.96pA, n=10; p = 0.026; Figure. 4D), while EE has no significant effect on the magnitude of feed-forward inhibition (Control: 567.3 ± 50.20pA, n=13; EE: 744.9 ± 109.8pA, n=12; p = 0.35). When we examined these parameters in details, we found that the increase of IPSC after cFC is mainly caused by the increase of PV-mediated FFI, as PV-mediated FFI significantly increased after cFC (Control: 165.4 ± 18.06 pA, n=13; cFC: 314.8 ± 40.21 pA, n=10; p=0.0014; Figure 4E) and CCK-mediated component was not sensitive to cFC (Figure. 4E and 4F). These results suggest the modulation effect of cFC on PV

inhibitory network not only change cellular excitability at single cell level, but also influence the feed-forward inhibitory transmission.

Comparing the IPSCs measured in control with those measured in preparations from environmental enrichment trained animals, we found that EE has no significant effect on the magnitude of feed-forward inhibition (Control: 567.3 ± 50.20 pA, n=13; EE: 744.9 ± 109.8 pA, n=12; p = 0.35, t-test). Interestingly, similar to the modulation to PV cells excitability, the FFI modulation by EE showed large variability as well, and existed subgroups of FFI on pyramidal cells. When examined the IPSCs in detail, we found there is a decrease in PV-mediated FFI after EE (Ctrl: 150.0 pA, n=13; EE: 102.3 pA, n=10; P=0.0371, Mann-Whitney test), which contributed to the decrease of total IPSC in one subgroup. CCK-mediated component was not sensitive to EE (Figure. 4E and 4F). These results suggest that EE probably influence the feed-forward inhibitory transmission via subgroups modulation.

Figure 4

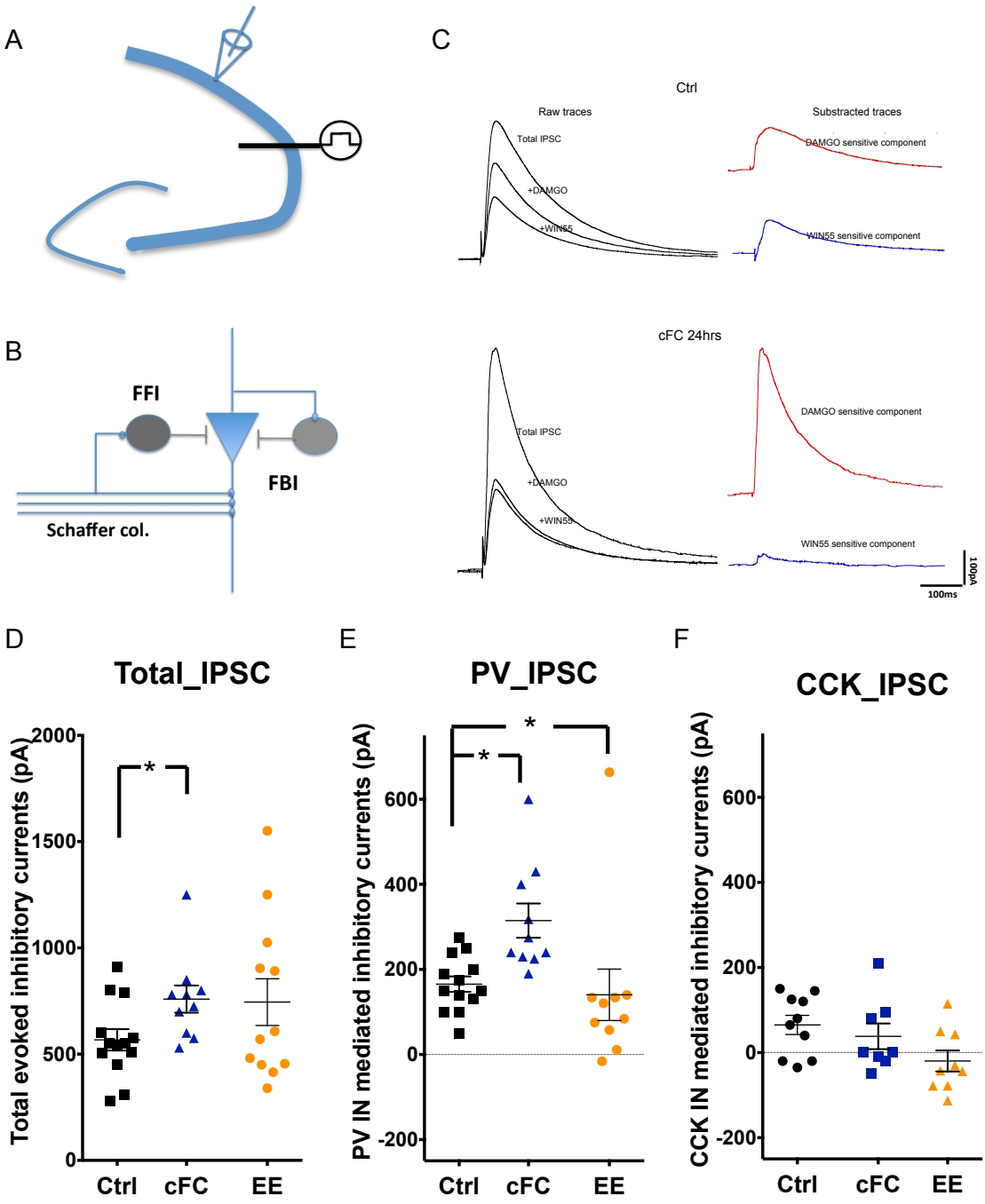

Figure 4: Experience modulate synaptic inhibition onto CA1 principal cells

A and B. Schematic graph of recording configuration. Stimulation electrode was placed at stratum radiatum between CA3 and CA1 to stimulate the Schaeffer collaterals fibres. Whole-cell recording were made on CA1 pyramidal cells to record direct excitation, di-synaptic feed-forward inhibition and tri-synaptic feedback inhibition.

C. Inhibitory synaptic currents traces from a voltage-clamped hippocampal CA1 pyramidal cell ($V_{holding}$=0 mV) in response to stimulation of the Schaeffer collaterals in caged control (upper) and in contextual fear conditioning (lower). Evoked IPSC onto the same pyramidal cell in the presence of the u-opioid receptor agonist DAMGO (100 nM) and subsequently in the presence of CB1 receptor agonist WIN55-212 (1 uM). Subtracted traces obtained before and after agonist wash-in. Red traces represent DAMGO sensitive inhibitory component (PV cell mediated), and blue traces represent WIN55-212 sensitive component (CCK cell mediated).

D-F. Summary graph of total IPSC, PV interneuron mediated evoked IPSC and CCK interneuron mediated evoked IPSC. All these three types of IPSC were measured in Ctrl (black squares), after cFC (blue triangles) and after EE (orange circles) conditions. The asterisk represents statistical significance ($p < 0.05$), error bars indicate SEM.

4. Correlation between WFA/PV expression level and firing frequencies in PV cells

Previous work from our lab showed that PV interneuron network is modulated by cFC and EE differentially and is shifted towards high-PV state or low-PV state (Donato et al., 2013). It is important to investigate whether the shifted expression level of PV indicate functional modulations as well.

A problem with PV expression measurement is that the PV amount changed after whole-cell recording. However, the peri-neuronal net (PNN) is well preserved after patch clamp. In Figure 6, we found a correlation between WFA and PV expression by quantifying WFA and PV fluorescence in intact cells (Figure. 6D, Ctrl: Pearson correlation r = 0.3483, p = 0.002; cFC: Pearson correlation r = 0.5159, p (two-tailed) < 0.0001). This data suggests high PV cells tend to have higher amount of peri-neuronal nets. Then we examined the correlation between WFA and firing rate in PV cells.

PV cells in acute slice were patched and filled with biocytin and the amount of PNN was calculated by WFA lectin binding activity in each experimental condition (Figure. 5A). Fast-spiking PV cells were then plotted with their WFA fluorescence activity and firing rates. In pooled form, data points linearly correlated (slope = 0.5234 ± 0.1697, n=63). Firing frequency and WFA intensity correlated significantly (Pearson correlation r=0.3964, p = 0.0033; Figure. 5B), suggesting high-WFA cells tend to fire at higher frequency. Considering the linkage between WFA and PV expression, it suggests that the high-PV cells may tend to fire at high frequencies. Therefore, the shift towards high PV expression after cFC suggest a higher firing frequencies in the PV cells.

In summary, high-PV expression level may predict a higher firing rate in PV cells and the shift toward high PV after cFC is consistent with increased firing rate as described in Figure 1.

Figure 5

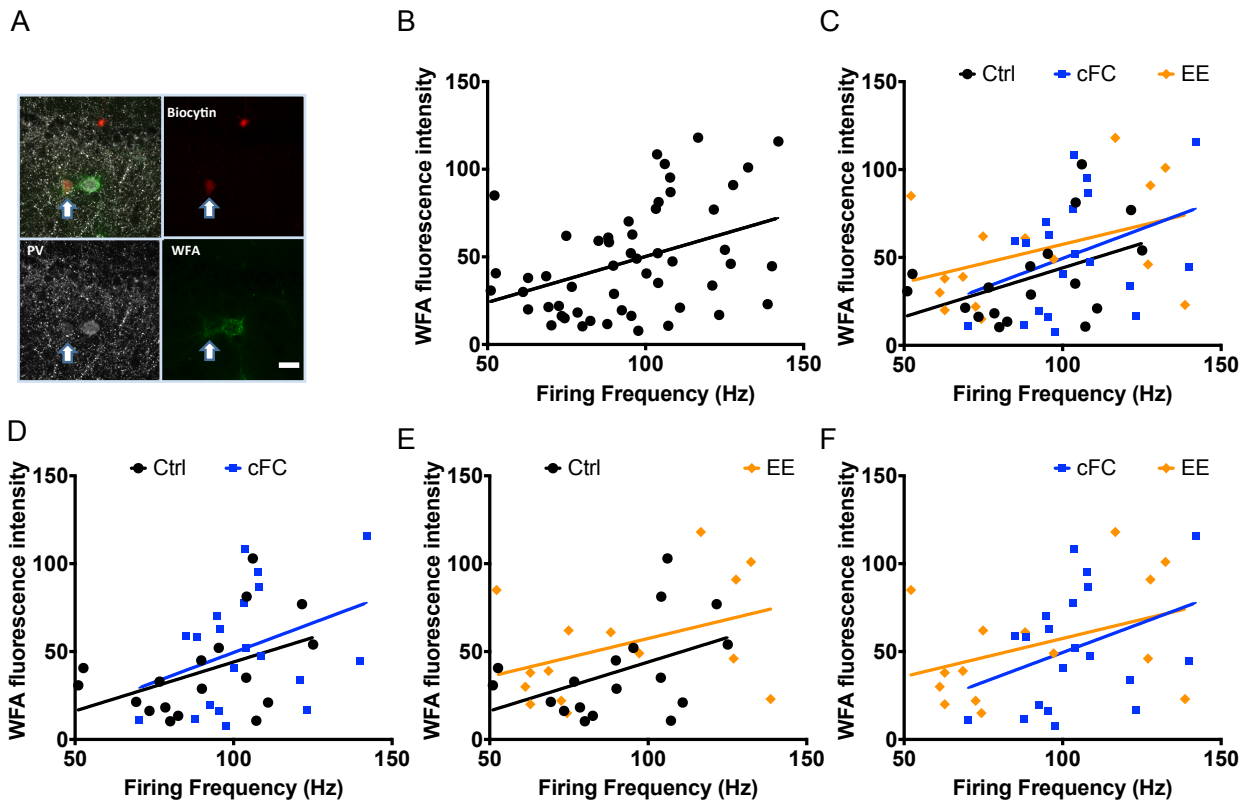

Figure 5: Experience modulation of PNNs in fast-spiking PV$^+$ cells in the adult hippocampal CA1

(A) Recorded neurons were filled with biocytin and subsequently were stained with parvalbumin and WFA lectin to reveal peri-neuronal nets (PNN). Biocytin in red; PV in grey; WFA lectin in green. The arrow indicated a recorded PV cells enwrapped by PNN from CA1 area. The upper right panel showed the distribution of WFA fluorescence intensity among Ctrl, cFC and EE. Statistical significance was determined by one-way ANOVA. There are no differences between each group (p value = 0.3675).

(B) Pair-wised scatter plot of firing frequency and WFA fluorescence intensity from pooled recorded PV cells. Best-fit slope is 0.5234 ± 0.1697, R^2 = 0.1571, Pearson correlation r = 0.3964, p = 0.0033 (two-tailed t test).

(C) Pair-wised scatter plot of firing frequency and WFA fluorescence intensity from PV cells in differential groups. Best-fit slope for control group is 0.5566 ± 0.2763, Pearson correlation r = 0.4498, p = 0.0611; Best-fit slope for cFC: 0.6739 ± 0.41583, Pearson correlation r = 0.3568, p = 0.1225; Best fit slope for EE: 0.4330 ± 0.2717, Pearson correlation r = 0.4042, p = 0.1351.

(D-F) Comparisons of scatter plot of firing frequency and WFA fluorescence intensity from PV cells in differential groups.

Figure 6

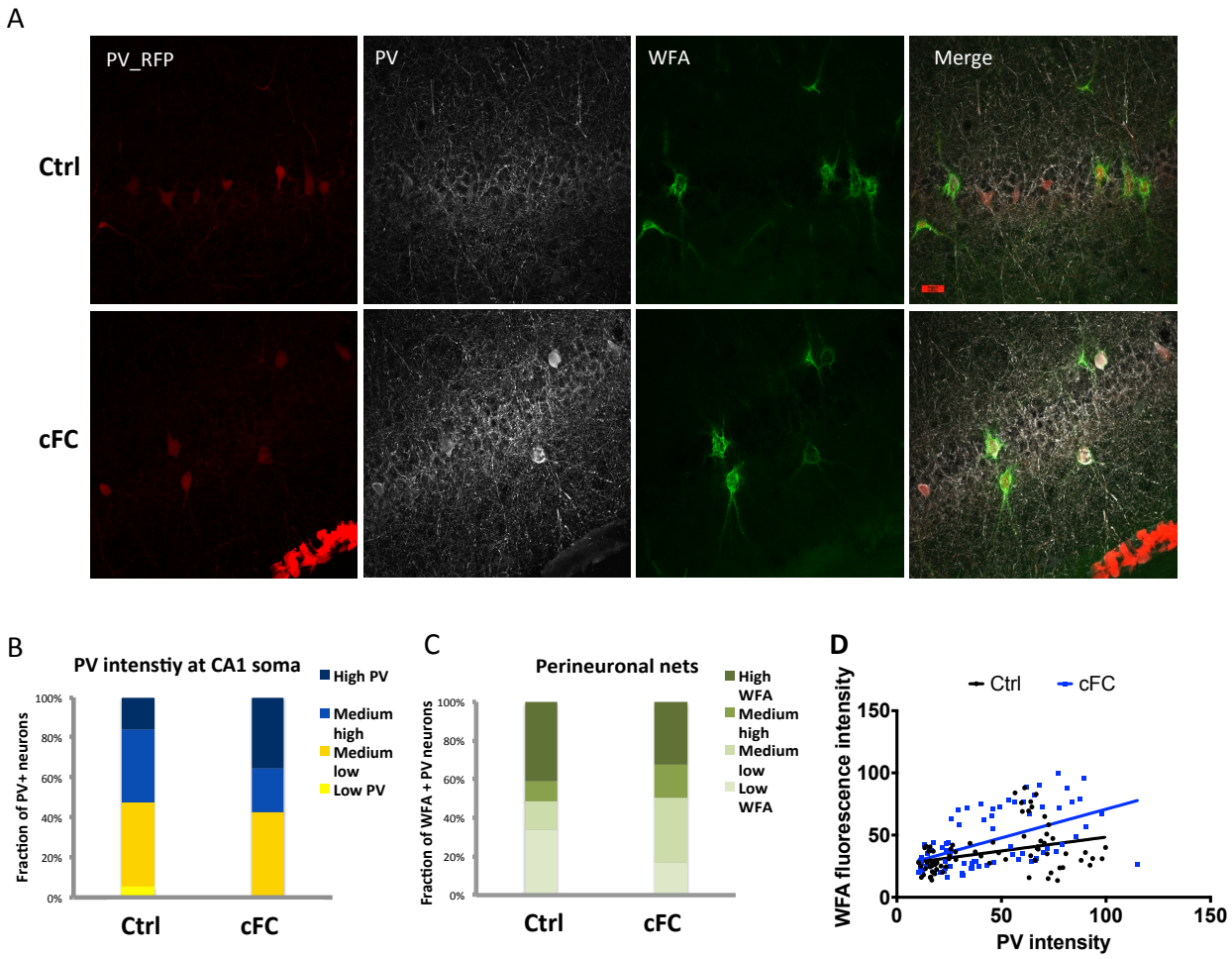

Figure 6 Modulation of PV and WFA expression in PV cells

A. Staining of PV and WFA in PV cells from caged control animal and animal trained with cFC. PV cells are transgenically labelled with RFP, and further immunostained with PV antibody (1:5000) in far red and binding with WFA lectin in green (1:500)

B. Fractions of high PV and low PV cells in the whole PV cell population. After cFC, the PV cell population shifted towards the direction of high PV expression.

C. Fraction of high WFA and low WFA cells in the whole PV cell population.

D. Scatter plot of WFA and PV fluorescence in control and cFC groups. There is a weak correlation between WFA and PV in control group. Such correlation gets strengthened after cFC training. (Ctrl, Pearson correlation r=0.3483, P=0.002; cFC, Pearson correlation r=0.5159, P (two-tailed)<0.0001).

3.4 Discussion

We reveal here an intrinsic neuronal plasticity in PV interneurons of the CA1 region upon experience. In addition to the previous findings that the synaptic modification of presynaptic connectivity onto PV interneurons can be induced by learning experience (Donato et al., 2013), the excitability of PV interneurons can be modulated by contextual fear conditioning and environmental enrichment. Moreover, contextual fear conditioning enhanced PV interneuron-mediated feed-forward inhibition in CA1 circuits; while environmental enrichment diversified its modulation on inhibitory transmission, and feed-forward inhibition provided by PV interneurons decreased in principal cells.

Modulation of intrinsic neuronal excitability of PV interneurons by experience

Previous studies from acute slice in juvenile rat showed that the intrinsic excitability of PV basket cells could be modulated by neural activity. High frequency stimulation of the Schaeffer collateral induced a persistent increase in the intrinsic excitability of PV basket cells in CA1 (Campanac et al., 2013). Here we provided direct evidence that behavior learning (24 hrs after contextual fear conditioning) facilitates the intrinsic excitability of fast-spiking PV interneurons in hippocampal CA1 of adult mice.

After contextual fear conditioning, we showed that the enhanced intrinsic excitability mainly reflects on higher firing frequency (Figure 1C), faster action potential kinetics (Figure 3A) and less frequency adaptation (sustained repetitive firing, Figure 2A,B). It is an important feature of PV interneurons that they are capable of responding to afferent inputs with action potentials of brief duration and firing repetitively at high frequencies. This ability to fire brief action potentials in rapid succession relies on action potentials that are non-decremental, of short duration, repolarize rapidly and possess brief after-hyperpolarizations and inter-spike intervals (Rudy and Mcbain, 2001). Therefore the changes of intrinsic excitability resulted from contextual fear conditioning represent a powerful means of recruiting PV^+ basket interneurons in hippocampal CA1 circuits.

After environmental enrichment training, the most significant change is that more PV interneurons firing adaptively, exhibiting more frequency adaption. However, their firing frequency tended to be diversified into two groups, with one group of PV neurons firing at higher frequency and the other firing at lower frequency compared with control group. Considering the result of PV mediated feed-forward inhibition on averaged group of CA1 principal cells, which showed again the diversified modulation, it suggested

that environmental enrichment influence PV interneuron network via subgroup modulation.

An intriguing question is that what could be the molecular mechanism underlying these changes as a result of behavioral learning. Several lines of evidence point to voltage-gated ion channels. The activity-dependent intrinsic neuronal plasticity relies upon the Kv1 channel activity (Campanac et al., 2013). In PV interneurons, Kv1 channels are mainly located on the cell body and on the proximal region of the axon (Wong et al., 1994). Regulation of the Kv1 channels substantially increases the excitability of fast-spiking interneurons by decreasing the voltage threshold for action potentials and increasing the near-threshold responsiveness (Li et al., 2012).

On the other hand, Kv3 channels are voltage-gated K^+ channels involved in the rapid repolarization of action potentials in neurons. They play a key role in the fast-spiking neuronal phenotype (Rudy and McBain, 2001). Indeed, Kv3.1 and Kv3.2 knockout mice show slower neuronal repolarization than wild type animals; Neurons in these knockout mice show impaired high-frequency firing (Porcello et al., 2002; Lau et al., 2000) and dysregulated gamma oscillations (Joho et al., 1999; Atzori et al., 2000). Meanwhile, lack of the intrinsic neuronal plasticity may constitute a cellular mechanism for neurological disorders in which the excitation-inhibition balance is altered (Lewis et al., 2012; Yu et al., 2006). A recent study showed that the expression of Kv3.1-containing K^+ channels is reduced in untreated schizophrenia patient and can be normalized with antipsychotic drugs (Yanagi et al., 2014)

Plasticity of intrinsic neuronal excitability in PV interneurons

What is the mechanism that learning regulates intrinsic neuronal excitability of PV interneurons? Long-lasting increase in excitability from persistent cell depolarization has been reported in basket cells of the dentate gyrus (Ross and Soltesz, 2001). Recent study has shown that high frequency stimulation of the Schaeffer collaterals induce a persistent increase in the intrinsic excitability of PV basket cells in CA1 within 30 minutes. (Campanac et al., 2013). These results suggest modulating depolarization of postsynaptic cells can be one potential mechanism for the induction of intrinsic plasticity. In current study, the changes of intrinsic neuronal excitability have long-lasting effects: 24hrs after fear memory acquisition or 3 weeks in environmental enrichment. For the long-term maintenance of intrinsic plasticity, can presynaptic arrangement be another contributor? Previous study showed that the density of excitatory synapses on PV interneurons was increased by contextual fear condition and the density of inhibitory synapses onto them was increased by environmental enrichment (Donato et al., 2013; Ruediger et al., 2011). The long-term effects can be supported by presynaptic rearrangement of

excitatory inputs onto PV cells: here after cFC, the increased input activity possibly lead to more depolarization in PV cells and subsequently be a contributor to the plasticity of intrinsic neuronal excitability of PV cells.

What is the biological significance of this altered intrinsic excitability of PV interneurons after learning experience? Considering that presynaptic connectivity also determines parvalbumin intensity (Donato et al., 2013), one aspect that would be fascinating to explore is that whether the enhanced intrinsic excitability underlies the change of parvalbumin intensity. PV interneuron network was bi-directionally adjusted by experiences: cFC shifted the network toward high PV state with larger fraction of high PV cells while EE shifted it toward low PV state with higher fraction of low PV cells. Previous research has shown how membrane depolarization and calcium influx in the cell enable to induce new transcription (West AE et al., 2001). Therefore, change of intrinsic excitability could be an outcome of structural change at presynaptic site and then transmitted the modification down to transcription level. High and low PV cells might be characterized by a set of differentially expressed transcripts and proteins that might underlie their functional significance.

Our study extends this view by showing the propensity of enhanced intrinsic excitability in high PV cells after cFC. The results from biocytin and perineuronal nets staining indicated that high firing frequency cells were usually surrounded by higher amount of perineuronal nets (Figure 2C). Considering the linear correlation between PV expression level and peri-neuronal nets, cells fire at high frequencies tend to be high PV cells (Figure 5B); thus the shift towards high PV expression after cFC is physiologically linked to higher firing frequencies in PV cells.

Modulation of parvalbumin interneuron mediated feed-forward inhibition upon learning

We showed here that contextual fear conditioning induced an increase in feed-forward inhibition provided by PV interneurons in averaged group of CA1 pyramidal neurons (Figure 4E). The enhanced recruitment of inhibition is likely to be achieved by increased synaptic recruitment and an increase in the intrinsic excitability of PV interneurons (Campanac et al., 2013; Result Fig 1C). On the contrary, environmental enrichment induced diverse changes in PV-mediated feed-forward inhibition similar to its modulation effect on intrinsic neuronal excitability (Figure 4E). Therefore the decreased recruitment of inhibition in one subgroup could result from the decreased firing rates in a subpopulation of PV interneurons (Figure 1C). Inhibitory transmission plays important roles in regulating the plasticity in the

microcircuit (Donato et al., 2013; Fagilini et al., 2004; Hensch 2005; Tian N et al., 1999;). Our results suggest contextual fear conditioning would increase feed-forward inhibition via PV interneurons to produce 'crystallized state' to reduce further learning, while environmental enrichment would decrease feed-forward inhibition via a subgroup of PV interneurons and produce 'plastic state' of enhanced learning.

This part of result focused on inhibition on averaged group of CA1 pyramidal cells. In this group of cells, the change of total inhibition (around 150pA, Figure 4D) after fear conditioning is mainly attributed to the change of PV-mediated feed-forward inhibition, while CCK interneurons did not exhibit significant effect (Figure 4F). However, the situation seems different after environmental enrichment. Although CCK interneuron did not exhibited any effect either, PV mediated feed-forward inhibition itself cannot account for the slightly increase of total inhibition (Figure 4D). These results suggest that EE affect the inhibitory circuit in a complicated way. It influences the feed-forward inhibitory transmission via PV subgroups modulation. Meanwhile, in addition to the perisomatic inhibition, other forms of inhibition like dendritic inhibition could also possibly be regulated by experience and contribute to the change of total inhibition.

4

Result II

Functional heterogeneity in hippocampal CA1 principal cells

4.1 Summary

Brain circuits show selective connectivity that is the structural basis for information processing (Brown& Hestrin, 2009; Yu et al., 2009). Recent studies from our lab demonstrated that there are at least two distinct subgroups of principal cells (Lsi1 and Lsi2) in mouse hippocampus. Each subpopulation of principal cells displays matched gene expression profile, matched neurogenesis and synaptogenesis time window, and prefer to interconnect within the subpopulation. However the neurophysiological properties of these subpopulations of cells still need to be elucidated and the functional difference among subpopulations of cells remains elusive.

In this study, we investigated the intrinsic properties and feed-forward inhibition onto these subpopulations of principal cells. Acute brain slices were obtained from juvenile (2-3 weeks) and adult mice (8 weeks) for electrophysiological recordings. Subpopulations of principal cells (Lsi1 or Lsi2) in hippocampal CA1, CA3 and DG were specifically targeted via transgenic reporter lines expressing membrane GFP under the control of neuron-specific Thy1 promoter respectively.

By whole-cell patch clamp recording, we showed that principal cells in CA1, CA3 and DG were not homogeneous. They exhibited wide range in firing frequencies, firing patterns, and intrinsic membrane properties. In hippocampal CA1, the spike amplitude is different between Lsi1 and Lsi2 subpopulations of cells: Lsi1 CA1 pyramidal cells fires significantly at larger amplitude, which may lead to stronger excitatory synaptic transmission. Interestingly, we observed changed firing pattern of principal cells as the animals mature. Both Lsi1 and Lsi2 subpopulations of cells showed enhanced adaptation and robust firing in adult stage. However, in both developmental stages, Lsi1 cells showed higher firing frequency in the initial phase of stimulation but adapted quickly in later phase compared with Lsi2 cells. These results suggest subpopulations of principal cells play differential roles in information processing in hippocampal CA1 circuits.

Then we further tested whether the modulatory effect of experience on feed-forward inhibition (FFI) depends on cell subtypes. We tested this hypothesis specifically in Lsi1 subpopulation. We found cFC modulated FFI on Lsi1 subpopulation of principal cells in the same direction as it did to averaged group of CA1 pyramidal cells. However, we found a larger variation in cFC group. In addition, EE enhanced the

total inhibitory postsynaptic currents (IPSC) significantly and slightly increased PV cell-mediated IPSC, which is different from averaged group of pyramidal cells in hippocampus. These results suggest the modulation of FFI by behavioural experience may be subpopulation specific.

Taken together, we show that subpopulations of principal cells exhibit distinct intrinsic electrophysiological properties that endow them to respond differently to learning experience (cFC or EE) by measuring inhibitory transmission in feed-forward connections onto them. This result also suggests that it is better to target a specific subpopulation rather than the averaged group of principal cells when giving a precise description of modulatory effect on the neural circuit by experience.

4.2 Introduction

An interesting feature of cortical circuit is the diversity of cell types involved in different biological functions. Plenty of studies have been done on different cell types of interneuron in hippocampus and cortex. Different subtypes of interneurons show distinct connections, molecular expression profiles, plasticity and functional features (Klausberger & Somogyi, 2008). Little is known about the identity and diversity of principal cells. Principal cells have often been tacitly assumed to be categorized into several major homogeneous populations in hippocampus (DG, CA3 and CA1). Recent findings from our laboratory have demonstrated that the existence of "microcircuits" in the hippocampus consisting of subpopulations of genetically-predefined principal neurons that are selectively interconnected as a result of temporally matched schedules of neurogenesis and synaptogenesis (Deguchi et al., 2011)., These evidence show that there could be more specifically defined subpopulations of principal cells, which may play distinct roles in neuronal processing or show distinct properties of plasticity and connection specificity (Brown & Hestrin, 2009; Yoshimura& Callaway, 2005).

Despite of the knowledge of the genetic profile and the development of subpopulations of cells in hippocampus, it is necessary to examine their basic neurophysiological properties and whether they respond differentially to learning experience. By using modified expression cassette based on the mouse Thy1.2 promoter, different subpopulations of principal neurons are labelled by two sparse Thy1 reporter lines (Lsi1 or Lsi2), which overexpress membrane-targeted GFP (mGFP) in a small proportion of principle neurons (De Paola et al., 2003; Gogolla et al., 2009). This enables us to examine their electrophysiological properties in acute brain slices and to investigate their distinct roles in the neural circuit after behavioural learning.

Studies linking molecular/cellular events to circuit function are important but also difficult. There are multiple routes to investigate the link between molecular and circuit level. One is to collect data at both levels simultaneously in a single set of experiment and describe the observations at different levels. However, the circuit is a complex component in information processing and it is not feasible to link its function directly to molecular level since individual cells function as a single unit during the process. Therefore, studies from the intermediate levels such as cellular and simple circuit levels by integrating the result of different levels from different experiments would be more plausible.

Intrinsic properties determine how a neuron responds to external inputs with its specific spatial and temporal profile. Neurons with high input resistance and strong adaptation usually respond to neural input in a fast and effective way, but such response fades easily when multiple inputs arrive in a short time window. On the other hand, cells with weak or no adaptation can respond to neural inputs constantly regardless of the frequency and magnitude of neural inputs. Previous studies on different subpopulations of principal cells have provided plenty of evidences on the specificity at genetic and synaptic levels (Donato et al., 2013; Lein et al., 2004; Thompson et al., 2008; Galimberti et al., 2010). The information of intrinsic properties could be very useful when interpreting the functional significance of these genetic, synaptic and connection specificities of different subpopulations.

Recent studies showed that excitatory connectivity onto PV-expressing interneurons in the hippocampus is also plastic and can be modified by contextual fear conditioning: the change in excitatory input shifts parvalbumin and GAD-67 expression to higher levels (Donato et al., 2013). These finding suggest that behavioural learning has a potent modulatory effect on the neural circuit involved. However, the functional consequences of such modulation at synaptic and cellular levels remain unclear. Meanwhile, how such modulation contributes to behavioural learning itself has to be understood in the context of circuit plasticity.

To better understand whether subpopulations of principle cells are involved in distinct inhibitory microcircuits, and whether their network plasticity depends upon experience, we systematically examined the intrinsic properties of different subpopulations of principal cells. The difference in their intrinsic properties is an important parameter for specifying different subpopulations, which also provides a basis to understand other subpopulation-related questions, such as how specific subpopulation of principal cells behaves upon experience at the circuitry level, and how is inhibitory transmission onto these cells modulated.

4.3 Result

1. Intrinsic properties of different subpopulation of principal cells in CA1 hippocampus.

Previous studies from our lab and other's work showed that principal cells in hippocampus are not homogenous but consist of different subpopulations. Principal cells can be preferentially interconnected to form specific microcircuits (Deguchi et al., 2011). To examine whether the subpopulations and their microcircuits have specific roles in information processing, we first tackle the neurophysiological properties of these subpopulations. Here we used the thy1-Lsi1 and thy1-Lsi2 line transgenic mice to visualize these two different subpopulations and selectively made whole cell recordings on these two subpopulations. We made systematic comparisons on intrinsic properties and firing pattern between these two different subpopulations. As shown in Table 1, seven key parameters of intrinsic properties were measured at two different ages and at three different site in hippocampus (2 weeks old and 8 weeks old in CA1 and DG; 2 weeks old and 3 weeks old in CA3): input resistance (Rm), spike amplitude (Amp), capacitance (Cm), spike half-width (t1/2), resting membrane potential (Vm), After-hyperpolarization potential (AHP) and spike threshold (Vt). These two subpopulations have similar results on most of these parameters, only that Lsi1 cells showed significantly larger spike amplitude in both CA1 and DG in adult mice (Table1, Lsi1, AMP=68.1±2.2mV; Lsi2, AMP= 62.8±1.6 mV, P=0.0451). Larger spike amplitude may lead to stronger synaptic transmission and suggest functional difference in the neural information processing in hippocampus. We further measured the firing pattern of these two subpopulations in CA1 hippocampus in 2 weeks old and 8 weeks old mice (Figure 1A). The firing patterns were classified based on their adaptation properties: single spike, strong adaptation (SA), weak spike adaptation (WSA) and weak spike adaptation with fatigue (WSA-F) (Figure 1B). As the animal mature, both Lsi1 and Lsi2 showed more adaptation and more robust firing (Figure 1C). At both ages, Lsi2 cells showed less adaptation than Lsi1, suggest that Lsi2 cells can respond to long lasting inputs better than Lsi1 cells (67.4% of SA firing pattern in Lsi1, while 25% in Lsi2). To further confirm what we observed from the traces of firing pattern, we plotted the firing frequencies of Lsi1 and Lsi2 in 100ms time bins for the whole 1s stimulus duration and fitted the trend of firing frequencies (Figure 2B). Consistent with results based on the traces of firing pattern, the exponential fit showed that Lsi1 cells fires at higher frequencies at the beginning of stimulation but adapted more quickly than Lsi2 cells. Besides firing pattern, the delay onset of first spike in response to rheobase current injection was also examined in Lsi1 and Lsi2 subpopulations of principal cells. Early onset was defined as onset time less than 100 ms.

We found WSA principal cells usually are late-onset cells, and Lsi2 cells consisted more late-onset cells (79%) while Lsi1 included larger fraction of early-onset SA (31%) (Figure 1D). Overall, these results showed that compared with Lsi2 cells, Lsi1 cells respond to afferent stimulus more quickly with higher firing frequencies and larger spike amplitude, while the response diminished more quickly. These differences suggest that these two different subpopulations play different roles in the neural information processing in hippocampal CA1 circuits.

Table 1

Intrinsic properties in hippocampal Lsi1 and Lsi2 subpopulations of principal cells (young and adult)

		CA1				CA3				DG			
		CA1_2w		CA1_8w		CA3_2w		CA3_3w		DG1_2w		DG_8w	
		Lsi1 (7)	Lsi2 (8)	Lsi1 (32)	Lsi2 (29)	Lsi1 (6)	Lsi2 (9)	Lsi1 (20)	Lsi2 (18)	Lsi1 (7)	Lsi2 (8)	Lsi1 (16)	Lsi2 (10)
Vm (mV)	Average	-63.1	-60.0	-64.2	-63.9	-63.3	-60.1	-66.3	-62.7	-74.8	-75.5	-77.5	-77.6
	Median	-64.0	-59.0	-64.0	-65.0	-65.5	-59.0	-67.0	-63.5	-77.0	-77.5	-76.0	-77.0
Vt (mV)	Average	-40.6	-39.5	-39.6	-38.5	-35.2	-28.2	-38.2	-39.6	-39.5	-31.0	-35.3	-31.7
	Median	-40.1	-40.8	-39.5	-38.9	-34.1	-28.7	-35.0	-43.1	-37.1	-35.1	-31.9	-31.8
Cm (pF)	Average	51.5	47.2	48.1	45.5	58.7	70.5	44.4	59.7	34.4	44.7	48.8	36.8
	Median	52.6	47.5	43.7	47.2	58.6	60.6	46.1	53.8	34.0	45.7	48.5	37.1
Rm (mV)	Average	161.2	175.5	162.3	147.2	116.5	100.6	150.3	121.9	390.9	245.0	185.9	214.4
	Median	153.5	169.1	150.3	140.5	105.2	109.7	136.3	131.2	268.6	235.4	174.7	192.8
fAHP (mV)	Average	-7.7	-8.3	-11.2	-10.3	*-3.9±2.4	-8.4±2.1	-10.6	-7.6	-12.9	-14.4	-14.7	-12.7
	Median	-8.2	-8.1	-10.2	-9.4	*-3.6	-7.4	-9.5	-6.4	-13.7	-13.5	-15.5	-12.3
Amp (mV)	Average	63.1	59.9	*68.1±2.2	62.8±1.6	79.1	74.5	59.7	58.0	51.2	50.1	*52.2±4.1	44.4±2.9
	Median	63.8	63.7	*69.2	64.0	78.0	78.0	53.3	54.0	52.8	53.5	*52.1	43.3
t1/2 (ms)	Average	3.0	3.3	2.8	2.9	*2.0±0.2	1.7±0.2	2.1	2.4	2.5	2.4	2.3	2.4
	Median	3.0	3.6	2.7	2.8	*1.9	1.8	2.1	2.3	2.6	2.3	2.2	2.3

Figure 1

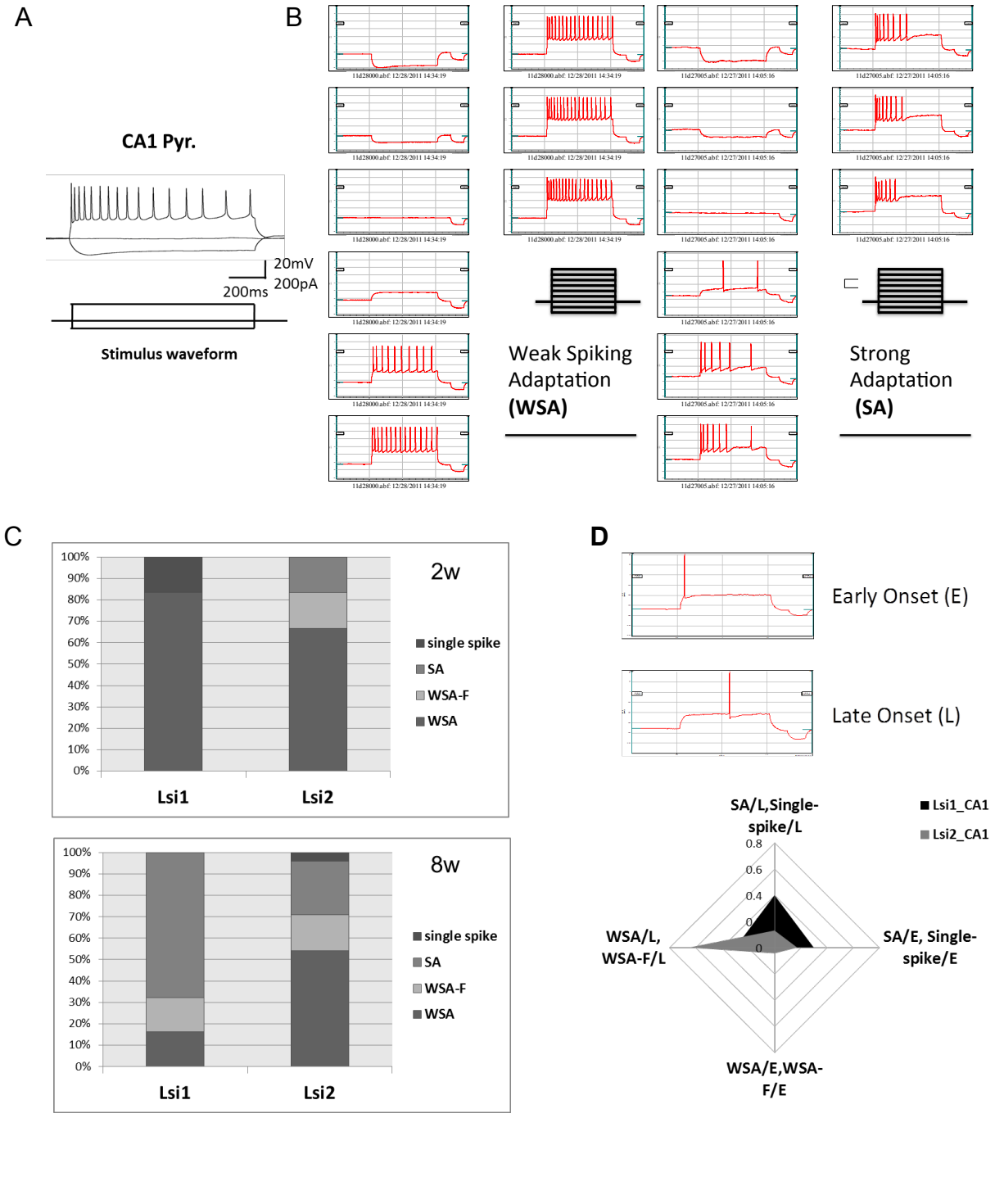
A
B
CA1 Pyr.
20mV
200pA
200ms
Stimulus waveform
Weak Spiking Adaptation (WSA)
Strong Adaptation (SA)
C
2w
8w
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
single spike
SA
WSA-F
WSA
Lsi1
Lsi2
D
Early Onset (E)
Late Onset (L)
SA/L,Single-spike/L
SA/E, Single-spike/E
WSA/E,WSA-F/E
WSA/L, WSA-F/L
Lsi1_CA1
Lsi2_CA1
0.8
0.6
0.4
0

Figure 1. Distribution of differential firing patterns in Lsi1 and Lsi2 subpopulation of CA1 principal cells

A. A typical firing pattern of CA1 principal cell in response to current injection.

B. Differential firing patterns among CA1 principal cells. Left: Weak spiking adaptation (WSA); Right: Strong spiking adaptation (SA);

C. Distribution of firing patterns in young (2 ws) and adult (8 ws) CA1 principal cells. In young mice, most principal cells are WSA for both subpopulations, while in adult mice, more cells show SA in Lsi1 and more cells show WSA in Lsi2.

D. Upper: Delay of firing by rheobase stimulation. There are two typical firing delays: early onset and late onset.
Lower: Radar distribution of combinations of firing pattern and firing onset time.

Figure 2

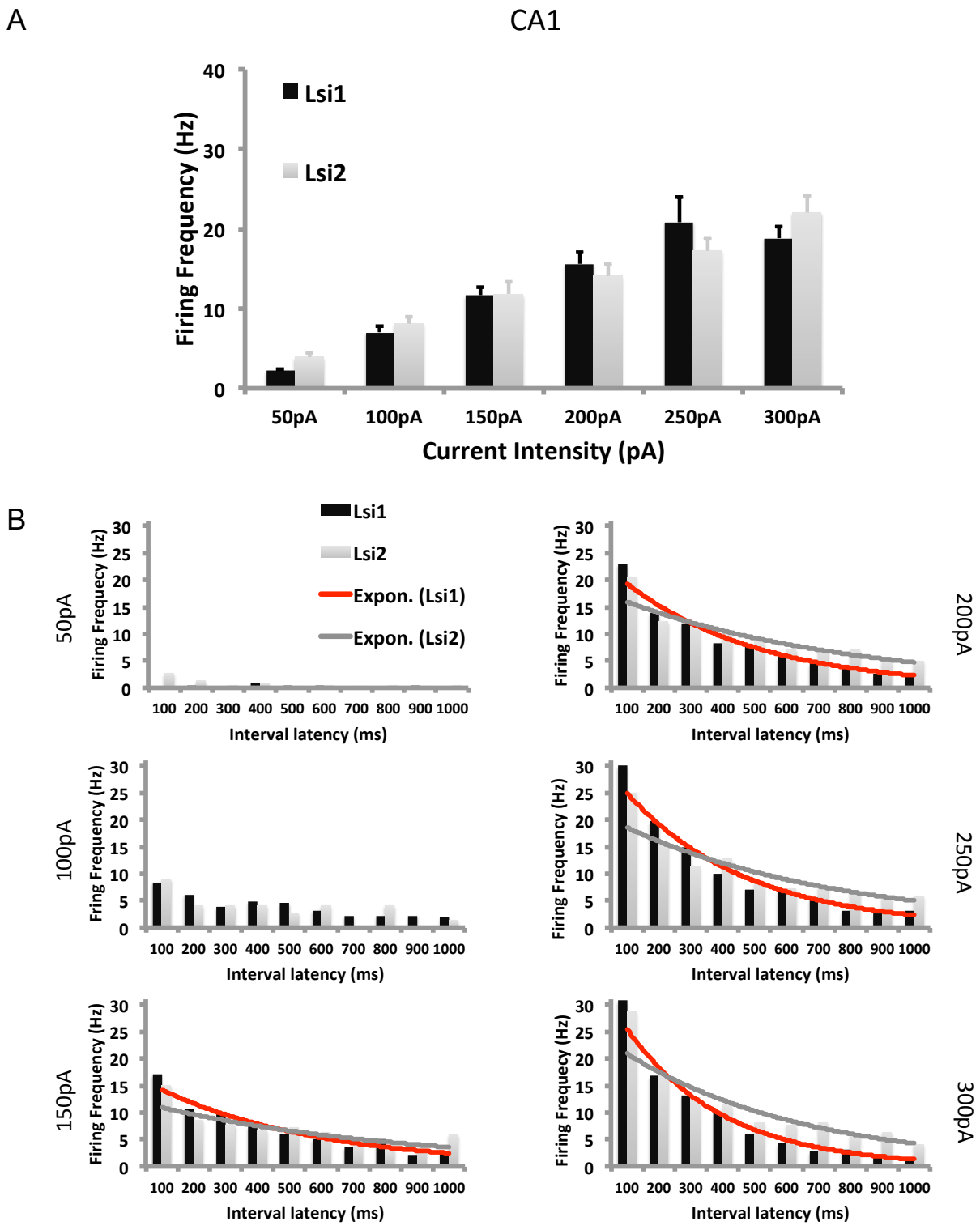
A
CA1
Lsi1
Lsi2
Firing Frequency (Hz)
40
30
20
10
0
50pA
100pA
150pA
200pA
250pA
300pA
Current Intensity (pA)
B
Lsi1
Lsi2
Expon. (Lsi1)
Expon. (Lsi2)
50pA
100pA
150pA
200pA
250pA
300pA
Firing Frequecy (Hz)
Fring Frequency (Hz)
Firing Frequency (Hz)
100 200 300 400 500 600 700 800 900 1000
Interval latency (ms)

Figure 2. Adaptation properties for different subpopulations of pyramidal cells in hippocampal CA1

Whole-cell patch clamp recordings were performed on hippocampal CA1 principal cells from transgenic Thy1-mGFP mice (Lsi1 & Lsi2).

A. Averaged firing frequency is analysed for both Lsi1 and Lsi2 within the whole course of depolarization pulse (1000 ms). The two subpopulations responded differently to the increase of injected current: Lsi1 showed adaption at 300 pA, whereas Lsi2 showed a linear increase as the injected current increased.

B. Averaged firing frequency calculated at 100 ms interval with differential amount of depolarization currents. The exponential fits of adaption curves show an initially higher firing rate but faster adaptation in Lsi1 (red) than Lsi2 (grey). Such temporal kinetics of firing property suggests different subpopulations have different intensity and duration of neural activity in response of same afferent input.

2. **Modulation of feed-forward inhibition onto Lsi1 cells by experience.**

The existence of different subpopulations requires us to consider the impact of heterogeneity in hippocampal principal cells. We have shown the recent experience can modulate the feed-forward inhibition in averaged population of CA1 pyramidal cells. Could the modulatory effect of experience on FFI depend on the identities of cells being measured? We tested this idea by examining the modulatory effect on FFI by recent experience in Lsi1 subpopulations. When measuring the FFI onto Lsi1 pyramidal cells, we found cFC exhibited the same modulation of FFI on Lsi1 subpopulation of principal cells as it did to averaged CA1 pyramidal cells. Both total IPSCs and PV cells mediated IPSCs increased with larger variations. (Control: 472.7 ± 56.53pA, n=12, cFC: 859.8 ± 113.2pA, n=14; t-test, P=0.0078). The increase of total IPSC can be partially explained by increase of PV mediated inhibition (Control: 148.3 ± 18.17pA, n=9;cFC: 249.9 ± 37.22pA, n=14; t-test, P=0.0286). Meanwhile, we observed a slight decrease of CCK cells mediated IPSCs (Control: 83.17 ± 32.78pA, n=12; cFC: 41.36 ± 37.84pA, n=14; t-test, P=0.4199). PV cells mediated feed-forward inhibition contributed most to the change of perisomatic inhibition and CCK mediated feed-back inhibition contribute little and only was changed in Lsi1 subpopulation. There must be other source of inhibitory inputs contributed to the increase of total IPSC on Lsi1 subpopulation after cFC other than PV and CCK cells. Interestingly, EE enhanced the total IPSC and slightly increased PV cell mediated IPSC (Figure 3A, B), which is different from what observed in the random chosen pyramidal cells (Chapter 3.3, Figure 4D,E). Total IPSCs increased unexpectedly and again with a larger variation (Control: 472.7 ± 56.53pA, n=12; EE: 1104 ± 141.0pA, n=10; t-test, P=0.0003). Although we observed the slight increase of PV cells mediated IPSCs (Control: 148.3 ± 18.17pA, n=9; EE: 286.3 ± 78.49, n=8; t-test, P=0.0968) and CCK cells mediated IPSCs (Control: 83.17 ± 32.78pA, n=12; EE: 153.0 ± 49.53pA, n=10; t-test, P=0.2399), the two perisomatic inhibitory cells seems not to be the sole contributor for the significant increase of total IPSCs. Taken together, the difference of the results between Lsi1 subpopulation and averaged pyramidal cell population suggest the modulation of FFI by behavioral experience may be subpopulation specific and the results for averaged pyramidal cells could be a mixture of different subpopulations.

Figure 3

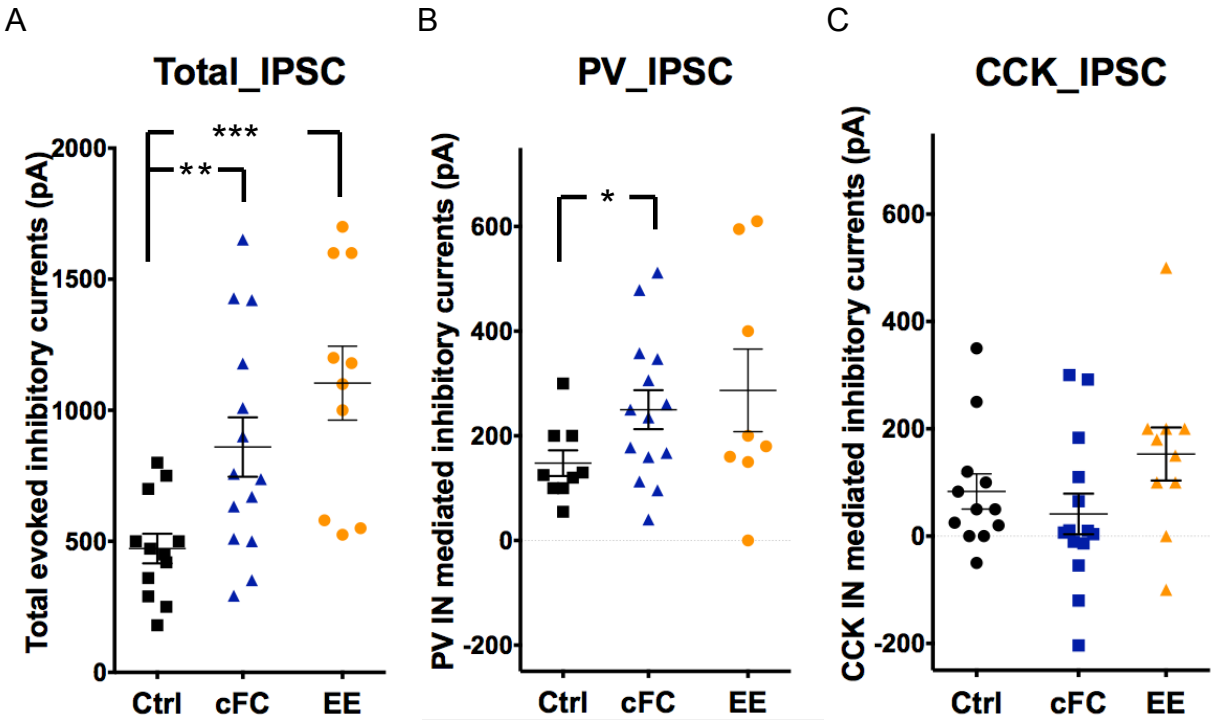

Figure 3 Modulation of feed-forward inhibition by experience in Lsi1 subpopulation of CA1 principal cells

A. Total feed-forward IPSC increased significantly after either cFC or EE training. Control: 472.7 ± 56.53pA, n=12; cFC: 859.8 ± 113.2pA, n=14 (P=0.0078); EE: 1104 ± 141.0pA, n=10 (P=0.0003).

B. The PV cell mediated IPSC increased significantly after either cFC or EE training. Control: 148.3 ± 18.17pA, n=9; cFC: 249.9 ± 37.22pA, n=14 (P=0.0286); EE: 286.3 ± 78.49pA, n=8 (P=0.0968).

C. The CCK cell mediated IPSC were not affect by cFC or EE training significantly. Control: 83.17 ± 32.78pA, n=12; cFC: 41.36 ± 37.84pA, n=14 (P=0.4199); EE: 153.0 ± 49.53pA, n=10 (P=0.2399).

4.4 Discussion

Based on the previous findings of genetically defined subpopulation in hippocampus, we have provided evidence for the difference in intrinsic properties between two subpopulations in CA1 hippocampus, the Lsi1 and Lsi2 CA1 principal cells, as well as the influence of subpopulation identity on circuit plasticity after behavioural learning. Such functional heterogeneity of CA1 principal cells suggest that at the cellular level, CA1 principal cells should be examined with better defined subgroups. Otherwise, the result from different subpopulations may be averaged out and the real computation in neural circuit may be masked.

Subpopulation-specific intrinsic neuronal excitability of principal cells in CA1

It has been shown that there is no significant difference between Lsi1 and Lsi2 subpopulations on most of indexes of intrinsic properties such as input resistance, resting membrane potential and spike threshold. However, these two subpopulations do differ in some important biophysical features including the spike amplitude and firing adaptation. The spike amplitude in Lsi1 is about 6 mV larger than that of Lsi2. The spike amplitude is a basic biophysical property since it could determine the propagation failure rate in the axonal fibres. Stronger spike amplitude will lead to less failure rate and more stable spike propagation into the synaptic boutons. Meanwhile, the release of neurotransmitter also depends on the spike amplitude. Larger spike amplitude suggests a stronger release of neurotransmitters in the synaptic terminals and larger synaptic potential in postsynaptic cells (Shu et al., 2006).

The impact of spike amplitude could be more significant considering the physiological environment when neurons are bombarded with hundreds of synaptic inputs per seconds in awake animals. The shunting current dampens both the spike prorogation and synaptic potentials. Larger spike amplitude in Lsi1 neurons suggests these cells could provide more stable synaptic input to postsynaptic cells.

Besides spike amplitude, the spike adaptation is also an important biophysical property which is determined by combination of inactivation properties of sodium and potassium channels. Lsi1 subpopulation show stronger adaptation than Lsi2 cells. The larger spike amplitude assures the stronger synaptic inputs from Lsi1 subpopulation, while the stronger spike adaptation means the synaptic input weakens quickly in a train of spikes and thus provide a precise temporal control.

Interestingly, the spike adaptation in pyramidal cells lead to higher firing frequency which is different from lower firing frequency in PV interneurons as a result of spike adaptation. Due to the stronger spike amplitude, spike adaptation and higher firing frequency, Lsi1 cells can give out stronger and more stable synaptic input at the beginning of a train of spikes with temporal precision since as the spike train persists, the impact by Lsi1 cells get weaker compared with Lsi2 cells. Such difference suggests different roles of Lsi1 and Lsi2 cells in the functional neural circuit: Lsi1 cells provide a strong synaptic input with precise timing and Lsi2 cells influence the postsynaptic cells through temporal summation.

Subpopulation –specific modulation of inhibitory transmission in CA1 circuit

Another feature we examined in subpopulations of principal cells is the feed-forward inhibition. Previous studies from our lab indicate that Lsi1 cells consist mostly of the earliest generated principal neurons in hippocampus among all the groups of subpopulations of principal cells. The electrophysiological properties of Lsi1 cells could probably stand out from the rest of subpopulations of principal cells. Our study of inhibitory transmission onto Lsi1 cells provided another piece of information about the functional specificity of this subpopulation of principal cells.

First it was shown that PV mediated feed-forward inhibition onto both averaged group of principal cells and Lsi1 cells can be enhanced by contextual fear conditioning; however, the environment enrichment decreased the PV mediated feed-forward inhibition in averaged group but in Lsi1 cells, the PV mediated FFI is largely unchanged and even slightly increased by EE (Figure 3B). The above result suggests the modulation of FFI by behavioural experience may be subpopulation specific, and the results for averaged group of principal cell could be a mixed consequence from different subpopulations. Such prediction can also be supported by the detailed examination of the PV-IPSC: the variation of FFI after EE training in averaged pyramidal cells group is similar to that of Lsi1 subpopulation, but Lsi1 neurons tend to have a larger proportion of cells with larger PV-IPSCs. To better understand the differential effects on averaged pyramidal cell group and Lsi1 subpopulation after EE modulation, further experiment on other subpopulations or a different behavioural learning paradigm need to be used to test this prediction.

Contextual fear conditioning also modulated Lsi1 subpopulation differently. In averaged group of CA1 pyramidal cells, change in PV mediated feed-forward inhibition could account for most of the change in total inhibition (increased around 150pA, Figure 4D, Result I) after conditioning since CCK interneuron did not exhibit significant contribution (Figure 4F, Result I). But in Lsi1 cells, more than half of the change in total inhibition cannot be explained as a result of change in PV mediated feed-forward inhibition. Therefore, different from the averaged group of principal cells, in which the modulation by cFC is mainly upon PV mediated FFI, the modulation of inhibitory inputs by cFC in Lsi1 subpopulation is upon more diverse targets, such as the dendritic inhibition mediated by cell types other than PV and CCK.

Another interesting point suggested by the comparison between averaged pyramidal cell population and Lsi1 subpopulation is that different behavioural learning paradigms may exert their modulatory effect on different groups of cells. The range of cells that a certain learning experience affects may reflect the nature of the behavioural content and the demand for neural computation. The larger variation of PV mediated FFI after EE suggested that EE affect the inhibitory circuit in a very complicated way: it influences the feed-forward inhibitory transmission via modulation on subgroup of neurons, which may reflect the complexity in the EE training paradigm.

In our study, the modulation effect of inhibitory transmission by behavioural learning depends on not only the learning paradigm itself, but also the group of principal cells we examined. To give a precise description of modulatory effect on the neural circuit by experience, it is better to target a specific subpopulation rather than the averaged principal cells. Besides, inhibitory transmissions of different origin can also be differential regulated, suggested that such modulation by behavioural learning can be quite precise and lead to a specific change in the inhibitory circuits.

5

General Discussion

General discussion

Plasticity of intrinsic neuronal excitability in behavioural learning

Plasticity in intrinsic neuronal excitability could underlie the formation of functional neuronal assemblies and may contribute to a specific memory trace (Debanne & Poo, 2010). The evidences for the linkage between intrinsic plasticity and learning come from both invertebrates (Alkon, 1984; Scholz and Byrne, 1987) and vertebrates (Moyer et al., 1996; Oh et al., 2003; Matthews et al., 2008). Recent work (Yiu et al., 2014) employing manipulation of voltage dependent K^+ channel to increase neuronal excitability in lateral amygdala showed that a higher excitability before training confers a competitive advantage for neuronal allocation to a fear memory. Our data on intrinsic excitability from Lsi1 and Lsi2 subpopulations of CA1 principal cells suggests Lsi1 cells are more likely to be recruited by learning. Lsi1 cells tend to fire with larger amplitude and higher firing frequency, thus their higher basal intrinsic excitability could endow them the first to catch up incoming inputs and encode memory.

In addition to principal cells, our results provide first evidence that learning experience could alter intrinsic neuronal plasticity in PV interneurons of the CA1 circuit. What properties do PV interneurons acquire and which would underlie plasticity of intrinsic neuronal excitability upon experience, thereby supporting learned behaviour?

Contextual fear conditioning enhanced intrinsic excitability in fast-spiking PV^+ interneurons by measuring the firing frequency. Concomitantly several other intrinsic properties changed: faster action potential kinetics, less frequency adaptation (sustained repetitive firing), reduced action potential amplitude and more hyperpolarized membrane potentials. How do these intrinsic properties work synergistically to produce enhanced intrinsic excitability? Together with the nature of low input resistance of PV cells, more hyperpolarized membrane potentials after cFC indicates that only strong synaptic inputs can activate these cells. Once they are activated, the smaller action potential amplitude, faster action potential kinetics and less frequency adaption will allow these interneurons to fire at higher frequency repetitively ensuring strong inhibitory control and precise timing in the complex neural network.

Functional interplay between experience-induced synaptic and intrinsic plasticity

Although intrinsic plasticity is about membrane properties and synaptic plastic is about synaptic weight, these two forms of plasticity are not independent to each other. Long-term synaptic potentiation is generally associated with increased neuronal excitability whereas synaptic depression accompanied by reduced intrinsic excitability (Daoudal and Debanne, 2003). Long-term synaptic plasticity and intrinsic plasticity share common induction protocol and expression pathways, which suggest they could be induced together by a certain learning experience.

Synaptic activation of glutamate receptors plays a central role in the induction of plasticity of intrinsic excitability in hippocampal neurons. Three major players are NMDARs (Wang et al., 2003; Campanac et al., 2008), mGluRs (Ireland and Abraham, 2002; Sourdet et al., 2003; Brager and Johnston, 2007) and kainate receptors (Melyan et al., 2004). Interestingly, PV^+ basket cells in hippocampal CA1 have recently been shown to undergo a long-term increase in intrinsic excitability after brief high frequency stimulation of SC inputs (Campanac et al., 2013). The increased excitability of PV cells is mediated by the activation of mGluR5 receptors because the phenomenon is diminished in the presence of a specific mGluR5 antagonist MPEP. Moreover, the intrinsic plasticity can be induced either by the direct application of the mGluR1/5 agonist DHPG or the specific mGluR5 agonist CHPG.

The induction mechanism for intrinsic plasticity of PV cells has not been tested in the present study. Previous study (Donato et al., 2013) has shown that by using the same behaviour paradigms, cFC shifts CA3 PV interneuron network to a high-PV-network configuration with increased density of excitatory synaptic punctas onto PV cells, and EE shifts towards a low-PV-network configuration with increased density of inhibitory synaptic punctas. In addition, in vivo transient inhibition of presynaptic inhibitory synaptic activity onto PV cells induced a robust shift to a high-PV-network configuration. We have shown here that 24 hours after cFC, the high PV cells are usually fast-spiking PV^+ cells with higher intrinsic excitability.

These results suggest that glutamate transmission and subsequent postsynaptic depolarization/hyperpolarization in PV cells is likely to play important roles in the long-term intrinsic plasticity induction and maintenance. In addition, previous literature has shown membrane depolarization and calcium influx in postsynaptic cells can induce new transcription (West AE et al., 2001). Alteration of intrinsic excitability is able to regulate gene transcription to support long-term

modifications on both synaptic and intrinsic modifications. The functional interplay between experience-induced synaptic and intrinsic plasticity thus can provide a way to ensure significant short-term and long-term changes in the neuronal output when plasticity is induced.

Subpopulation-specific modulation of inhibitory transmission

We have shown that the level of basal intrinsic excitability in Lsi1 subpopulations of principle cells is different from Lsi2 cells in CA1, and Lsi1 cells tend to spike with larger AP amplitude at higher firing frequencies but with stronger adaptation. The inhibition onto Lsi1 cells has also been shown with differential recruitment upon learning.

A widespread increment of PV cell-mediated feed-forward inhibition (PV-FFI) was observed in both Lis1 and averaged population of pyramidal cells after cFC. In both averaged group of principle cells and Lsi1 cells, CCK cell-mediated feedback inhibition (CCK-FBI) was unchanged. Interestingly, cFC led to a larger increase in total inhibition onto Lsi1 CA1 cells and the change of PV-FFI could not solely account for this increase. Since the PV and CCK cells are the major sources of somatic inhibition, the difference between the total IPSC and the sum of PV and CCK mediated IPSC can be largely attributed to inhibition with dendritic origin. Further calculation of the potential dendritic inhibition suggested that the dendritic inhibition of Lsi1 cells may be specifically recruited upon fear conditioning. The enhanced dendritic inhibition could result from increased SOM interneuron excitability (Mckay et al., 2013). EE exhibited its modulation on Lsi1 cells in a different way. Instead of relieving the pyramidal cells from perisomatic inhibition, both PV-FFI and CCK-FBI was slightly increased in Lsi1 cells by 3 weeks of EE and further contributed to the significant increase of total Inhibitory postsynaptic currents.

Previous study has indicated that the specificity in innervation of different subcellular domains in principal cells has profound consequences on the function that interneurons exert in cortical microcircuits (Miles et al., 1996). Somatic inhibition by PV cells and CCK cells is likely to control the output of principal cells and is important for the synchrony of action potentials of large populations of cells. Other sources of inhibition such as the dendritic inhibition could be responsible for the control of the efficacy and plasticity of glutamatergic inputs from specific sources (Freund and Katona, 2007). Our work therefore provides a potential circuit mechanism for differential recruitments of inhibition by distinct experiences in a subpopulation-specific manner. The cFC mainly enhanced the inhibition control from PV cells onto the whole principal cell populations while for Lsi1 subpopulation, inhibition from origins other than PV and CCK cells is also enhanced by cFC; EE relieved most principle cells from PV

mediated somatic inhibition and shifted the neuronal network to a plastic state, while interestingly kept the PV mediated inhibition onto Lsi1 subpopulation of principal cells largely unaffected.

Final Remarks

From the neurophysiological point of view, my thesis work provided direct evidences for experienced induced plasticity of intrinsic neuronal excitability in PV interneurons and of PV mediated feed-forward inhibition in the context of an adult microcircuit. The results showed that behavioural learning could exert functional modulation in PV interneuron network at both cellular and circuit level. Both forms of plasticity are long-term effects, and training paradigm specific. Furthermore, taking the advantage of genetically defined subpopulation in hippocampus, we tested the hypothesis of the subpopulation-specific recruitment of inhibition upon experience and provided preliminary results for this intriguing arrangement for experience induced circuit plasticity.

Long-term synaptic plasticity has long been assumed as the neural mechanism underling learning and memory. Recent studies on the intrinsic plasticity showed that not only the synaptic connection but also the neural excitability could be the target of modulation. Considering that contextual fear conditioning leads to a high-PV configuration and environmental enrichment leads to a low-PV configuration, it will be important to examine the causal relationship between intrinsic neuronal excitability and differentiation state of PV cells.

Our work is just a beginning of understanding how behavioural learning changes inhibitory circuitry. There are still many important questions to be answered. For example, what is the cellular and molecular mechanism for the changes in intrinsic excitability of PV cells? How does neuromodulators influence the inhibitory circuit? Considering the emotional involvement differing in behavioural paradigms, how could emotion impact on interneuron network? These would help to answer some basic questions in neuropsychiatric disorder since many of them relating to the dysfunctions of emotion regulation.

5

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