Cellular- and network-level regulation of place cell activity mediated by activity-dependent gene, Arc

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**Cellular- and network-level regulation of place cell activity
mediated by activity-dependent gene, Arc**

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School of Biological Science

2019

Cellular- and network-level regulation of place cell activity
mediated by activity-dependent gene, Arc

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A thesis submitted to Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Doctor of Philosophy

2019

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Summary

Immediate early genes (IEGs) have been suggested to be an important mediator of activity-dependent changes in the brain and are used as biomarkers to label memory engrams. One immediate early gene, activity-regulated cytoskeleton-associated protein (Arc), bridges experience to synaptic modification. Arc functions as a master regulator of many forms of synaptic plasticity, which is engaged in information encoding and storage. How Arc modifies neuronal activity associated with experience is unknown.

We have used hippocampal pyramidal neurons as a model to study the function of Arc in modifying neuronal activity in the experiment described in this thesis. Some of hippocampal pyramidal neurons exhibit location specific firing patterns, and thus are known as place cells. We knocked down Arc in a small portion of hippocampal CA1 area and recorded firing activities of pyramidal neurons in freely behaving rats to investigate how it changes neuronal activity.

We found that both control and Arc knock down neurons showed place cell activity. However, Arc knock down place cells showed significantly lower firing rate and more specific spatial tuning patterns. Further detailed analysis revealed that this reduction was more extensive outside of place fields than inside. This biased firing rate reduction outside of place fields resulted in increased spatial specificity. In addition, we found that spikes from Arc knock down pyramidal neurons show stronger phase-locking to theta

oscillations than control, which is considered to be a narrower temporal tuning. Next, we asked the consequence in neuronal population properties. Arc knockdown neurons did not fire together as frequently as control neurons, indicating that they are less likely to interact with each other and simultaneously activating common postsynaptic neurons. Further, we evaluated how accurately the activity of neuronal population can represent animal locations. Arc knockdown groups made larger error in estimated locations compared with control group, which suggests that Arc knockdown worsens the spatial representing ability of neuronal population.

Our result suggests that Arc increases firing rate and expands the tuning of spatiotemporal neuronal firing activity at the cellular level, which improves population functions. This cellular- and network-level firing patterns regulated by an experience-induced gene might contribute to the formation of memory engram.

I. Introduction

I.1 Immediate early gene defines memory engram

Learning and memory induces activity-dependent persistent modifications in the brain, which is thought to underlie memory storage [1] [2] [3]. These activity-dependent changes are summarized in a term, memory engram. The idea of memory engram was introduced in 1904 by Richard Semon as ‘the enduring though primarily latent modification in the irritable substance produced by a stimulus [1]. Combining the current knowledge of nervous system, the engram has been defined in contemporary contexts as ‘the enduring physical and/or chemical changes that were elicited by learning and underlie the newly formed memory associations [4].

Studies have mapped engrams in different scales. Early lesion or stimulation studies led to the conclusion that some types of memories are associated with brain regions [5] [6] [7]. This conclusion was elaborated by the combination of behavioral paradigms and electrophysiological or fMRI studies which indicates that memory engram should be located in those brain regions associated with the memory [8] [9].

What is the basic unit of memory engram? The most widely accepted view is the memory engram lies at the synapse level. In 1949, Donald Hebb hypothesized that the formation of cell assemblies by enhancing the neuronal connection if neurons fire together [10]. This hypothesis was supported by the discovery of long term synaptic plasticity: long-term potentiation (LTP) in the rabbit hippocampus [11] and long-term depression (LTD) in the rat hippocampus [12]. LTP and LTD are the persistent

strengthening or weakening of synaptic strength, respectively, which are caused by physical and chemical changes in the properties of synapses such as the number of ionotropic transmitter receptors and the size and shape of synaptic structures. Synaptic plasticity modifies neuronal firing patterns [13] and influence other neurons through synaptic connections in the network [14]. This functional change at the neuronal then network level are thought to underlie memory formation and storage.

Recent studies successfully established a method to identify a set of neurons which make a major contribution to memory formation, storage and retrieval [15]. In this study, they achieved a genetic method to label neurons activated during contextual fear conditioning by the expression of channelrhodopsin-2. Later optical activation of these labeled neurons resulted in appearance of freezing behavior, which is an indication of fear memory retrieval. In this way, they showed that artificially inducing the firing of a group of engram cells is sufficient to make animals recall memory [15]. These neurons were named engram cells, and a series of studies established that engram cells get activated during learning and are reactivated during memory recall and are required for memory recall [16] [17] [15]. These characteristics of engram cells indicate that memory engram should exist inside engram cells.

In line with this idea, studies [18] found that fear conditioning induces synaptic plasticity in engram cells. Nanoka et al. found enhanced transmitter release efficacy in basolateral amygdala neurons recruited into memory engram [19]. Another found that engram cells in dentate gyrus have higher spine density and synaptic strength [18]. A recent study

further elaborated these findings by showing changes of synapse density and size in engram cells selectively happened in those synapses formed by other engram cells [14]. They genetically labeled CA3 and CA1 engram cells with different fluorescent markers and visualized synapses formed between CA3 and CA1 engram cells. They found that these synapses have larger size higher density and better transmission efficacy than other synapses. The enhanced synaptic connections tie engram neurons together to play a role in memory retrieval [18].

In most of previous studies, engram cells have been genetically defined by using immediate early genes (IEGs) promoter [15] [20]. Immediate early genes (IEGs) are activity-dependent molecules which rapidly express in activated neurons [21] [22]. Many of them have been identified as a critical component in synaptic plasticity and memory [22] [23]. Although these studies use the property of immediate early genes only for labeling purposes, it is also important to consider how these immediate early gene, whose expression is induced during memory formation and retrieval, contribute to those memory processes. Considering that synaptic plasticity is widely believed as a major type of memory engram, we are particularly interested in immediate early gene which regulates synaptic plasticity. One of such genes is activity-regulated cytoskeleton-associated protein (Arc or Arg 3.1, detailed explanation from section I.2) [24] [21], which is considered as a master regulator of synaptic plasticity [25].

I.2 Activity-regulated cytoskeleton-associated protein (Arc/Arg 3.1)

In recent years, immediate early genes (IEGs) have been widely used as biomarkers for engram cells [4], due to low resting expression level and fast selective expression following neuronal activity.

The IEG products include secreted proteins, transcription factors, and cytoskeleton-associated proteins [22]. The gene that we are interested in is called activity-regulated cytoskeleton-associated protein (Arc or also called Arg 3.1) [24] [21]. Arc has been identified as a critical component in protein synthesis dependent synaptic plasticity and memory [25] [23]. The manipulations of Arc abolish normal functions from the synaptic to behavioral level [26]. This leads us to consider that it may not be only a biomarker for engram cells, but also be a part of mechanism underlying memory engrams.

I.2.1 Arc structure

Arc protein is 396 amino acid long (Figure I-1). It is only found in vertebrates but homologs are found in invertebrates. It has a coiled-coil domain [amino acid (aa) 28-75], an N-lobe (aa 207-278), and a C-lobe (aa 278-370) which contains a spectrin homology domain. The N-lobe and C-lobe together form the Arc Gag domain [27], which has homology to retroviral group-specific antigen (Gag) protein forming viral capsid structure [28]. In addition, there are a nuclear retention domain (NRD, aa 29-78), a nuclear export signal (NES, aa 121-154) and a nuclear localization signal (NLS, aa 331-335) [29] for the regulation of nuclear localization. Arc also contains binding sites to endophilin 3 (aa 89-100), dynamin 2 (aa 195-214) [30], and clathrin-adaptor protein 2 (AP-2, aa 195-199) [31], which were reported to mediate α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) endocytosis [32].

Figure I-1: Arc protein structure. Arc contains a coiled-coil domain (CC, aa 28-75), nuclear retention domain (NRD, aa 29-78), nuclear export signal (NES, aa 121-154), nuclear localization domain (NLD, aa 331-335), binding sites to endophilin 3 (Endo 3, aa 89-100), clathrin-adaptor protein 2 (AP-2, aa 195-199), and dynamin 2 (aa 195-214). The N-lobe (aa 207-278) and C-lobe (aa 278-370) of form the Arc Gag domain.

1.2.2 Arc transcription, transportation and translation

Arc mRNA is rapidly expressed in the neurons in response to neuronal activities and LTP induction. The mRNA can be detected within 5 mins when a rat explores a novel environment [33]. Although the signaling cascades from neuronal activity to Arc transcription are not fully understood, it has been reported that an activity-dependent transcription factor, myocyte enhancer factor-2 (MEF2), upregulates Arc expression [34] [35]. Two enhancer regions ~6.5 kb and ~1.4 kb upstream of Arc have highly conserved serum response elements (SRE) which binds to transcription factors serum response factor (SRF) [36] and regulates Arc transcription in an NMDAR-dependent manner.

Arc mRNA is transported to cytoplasm within 30 min of transcription in CA1 neurons[37] and can be found in dendrites within 1-2 hour of transcription in dentate gyrus [38]. In addition to fast dendritic targeting, a large fraction of Arc mRNA was also detected in the nucleus [29]. Arc translation is also highly dependent on neuronal activity. Bloomer et al. showed in cultured hippocampal neurons, activation of NMDA receptor is required for efficient Arc translation, and further enhancement can be regulated by the activation of Gs-coupled dopamine or β -adrenergic receptors [39]. Extracellular-signal-regulated kinase (ERK) through MAP kinase-interacting kinase (MNK) signaling was reported to be required for enhancing Arc protein synthesis in vivo after LTP induction [40]. Mirroring the mRNA localization, Arc translation sites are in cell body and throughout the whole dendritic laminae, but highly dense in cell body and activated dendritic area [41] [42]. Arc protein regulates AMPAR endocytosis and contributes to different forms of synaptic

plasticity (this part is expanded in following section). The localized Arc synthesis in activated dendritic area thus supports synaptic changes following neuronal activities.

I.2.3 Arc regulates AMPAR endocytosis

AMPARs are ionotropic transmembrane receptors for glutamate [43]. They are highly mobile and can be inserted into and removed from synapses through exocytosis and endocytosis, respectively [44]. AMPAR exocytosis inserts the receptors into the postsynaptic membrane to increase synaptic strength [45], while AMPAR endocytosis removed the receptors from the membrane to weaken it [46]. Arc protein positively regulates the AMPAR endocytosis and thus decrease synaptic strength, through interacting with endophilin 3, dynamin 2 and clathrin-adaptor protein 2 (AP-2) [30] [31]. Arc protein binds to AP-2 to initiate the endocytic vesicle cargo [31]. It then binds to dynamin and endophilin, which work as scissors to release the AMPAR endocytic vesicles from the membrane [47] [48] [49]. Manipulations of Arc confirmed its significant role in AMPAR trafficking and synapse strength modification. In Arc knockout mice, neuronal membrane surface AMPAR level is significantly higher than wildtypes [30]. As a consequence of facilitating APMAR internalization, Rial et al. further showed increase in Arc expression reduces AMPARs dependent synaptic transmission [32].

I.2.4 Arc mediates inverse synaptic tagging

Maintaining long-term synaptic change requires plasticity related proteins to either be translated locally or transported from cell body and dendrites [50]. However, mRNA transcription and most protein synthesis do not occur locally in activated spines. How do the mRNA and synthesized proteins recognize activated spines and specifically transport and contribute to them? A proposed mechanism is synaptic tagging (Figure I-2), a process by which synapses establish 'tags' early after potentiation to later allow them to capture newly synthesized proteins or mRNA which could be locally translated, to maintain a long-lasting synaptic change [51].

Figure I-2: Synaptic tagging. Upon stimulation, activated synapses (red) develop tags. These tags differentiate activated (red) and non-activated (black) synapses. The tags help plasticity related proteins and mRNAs be captured to maintain the increased synaptic weights.

Arc was thought to tag activated synapses. With light microscopy, a study showed that, after stimulating synapses located at a layer of the dentate gyrus (Figure I-3A), Arc mRNA

accumulates in the dendritic area within the activated layers (Figure I-3B) [38]. An electron microscopy study [52] using the same stimulation method further showed that Arc proteins amount is higher in spines located in the stimulated layer than non-activated layers. The idea that Arc tags activated spines was drawn from these studies, although no direct evidence showed the spines which contain Arc are activated spines.

Figure I-3: Arc mRNA selectively localized in stimulated dendritic segment. **A.** Dentate gyrus molecular layer stimulation. Presynaptic terminals from the medial entorhinal cortex selectively target the middle molecular layer (MML) of the dentate gyrus. By activating entorhinal cortex, a band of synapses are activated in MML of dentate gyrus. GCL, granule cell layer; IML, inner molecular layer; MML, middle molecular layer; OML, outer molecular layer. **B.** After 2 hours of stimulation, Arc mRNA were detected in GCL and MML. Figure on the right is a schematic illustration of Arc mRNA distribution in granule cells. It shows that, in addition to cell body and nucleus, Arc mRNA transportation selectively targets activated MML dendritic regions. (Adapted from [38]. Copyright © 1998 Cell Press.)

The idea of Arc targeting to potentiated synapses conflicts with the finding that Arc in synapses facilitates AMPAR endocytosis and reduces synaptic transmission [30] [32]. This conflict was resolved by the finding that Arc is accumulated in inactive or less active synapses following neuronal activation [53]. This inactive synapse targeting was named inverse synaptic tagging. Arc preferentially binds to an inactive form of CaMKII β and

localizes in inactive spines. The inverse synaptic tagging by Arc is thought to decrease the synaptic strength of inactive synapses following plasticity-inducing stimulation. By weakening inactive synapses, the contrast between strong and weak synapses becomes larger.

Steward et al's work, together with other studies, showed large fraction of Arc mRNA and protein are accumulated in the dendritic area near strongly activated synapses following LTP induction. Arc knockout models showed a deficit in LTP and in spine morphologies following the same protocol of stimulation. These observations helped to draw the conclusion that Arc mRNA is accumulated in the dendrites near where synapses are activated and contribute to the potentiation. However, these studies did not answer whether Arc directly regulate the activated synapses.

In Okuno et al paper, they found with BDNF application, Arc mRNA binds to the inactive form of CaMKII β and selectively localized in non-activated synapses. This study showed that Arc transcription requires neuronal activity, and localization in synapses targets to weak or inactive synapses.

This two findings do not contradict with each other. Both of them showed neural activity triggers Arc transcription. For localization, Arc accumulates activated parts of dendrites. In the activated part of dendrites, Arc targets weak and inactive synapses to presumably eliminate these synapses. Taking these findings together, Arc helps to keep the activity induced change in synapses.

I.2.5 Arc and synaptic plasticity

Regulation of dendritic spine structure

The spectrin homology domain in Arc gives a hint of its importance in spine morphology. Spectrin is a cytoskeletal protein which is crucial for maintaining the structure of a cell membrane and the shape of the cell. It mainly interacts with actin, ankyrin and lipids [54]. The homology with spectrin allows Arc protein to interact with actin. The application of Arc antisense oligodeoxynucleotides results in loss of F-actin at synaptic spines. The structural change in spines depends on the accumulation of F-actin as well as new protein synthesis [21]. Arc overexpression in cultured neurons leads to the reduction of stubby spines but increase of thin spines and filopodia. In Arc knockout mice, lower spine density and increased spine width were observed in CA1 and DG neurons [55]. Altogether, these finding suggests that Arc supports the maintenance or generation of spines with narrower width. These spines are thought to be 'learning spines' because they are more motile and transient and have greater capacity to enlarge and stabilize after LTP [56]. Thus, Arc expression might improve the learning ability by making neurons ready to participate in memory engram.

Long-term synaptic plasticity

When Arc antisense oligodeoxynucleotides was injected into the hippocampus to inhibit Arc expression before electrical stimulation inducing LTP, the maintenance of LTP was impaired without affecting the induction of LTP [57]. In Arc knockout mice, the early phase of LTP was enhanced but the enhanced synaptic efficacy decreased quickly, both

in dentate gyrus in vivo and in CA1 in brain slices [58]. These results indicate Arc is needed to maintain the enhanced synaptic transmission but not for inducing this potentiation.

In addition to LTP, facilitation of AMPAR endocytosis links Arc function to NMDAR-dependent LTD. Arc knockout or knockdown mice have abolished this type of LTD because of reduced AMPAR internalizing rate [59] [60].

Homeostatic plasticity

Homeostatic plasticity refers to a phenomenon that neurons adjust their own activity in response to prolonged changes in input activity [61]. It scales synaptic efficacy of the whole neuron, or a subcellular region, to a direction opposite to the change in input activity, without changing relative strength between involved synapses [62] [63].

Synaptic AMPARs levels get upregulated by chronic inactivity of a neuron while downregulated by constant increase in activity [64] [65]. This homeostatic like AMPAR regulation is abolished by either Arc overexpression or knockout [66] [67]. It was not clear how Arc regulates this cell-wide or subcellular region-wide scaling until a study in 2013 found nuclear localized Arc blocks GluA1 (AMPA subunit) transcription [29]. It showed Arc overexpression promotes the formation of promyelocytic leukemia nuclear bodies, which suppress GluA1 transcription.

I.2.6 Arc function in behavior and memory

When animals are exposed to a novel environment repeatedly, Arc transcription was detected in a highly overlapping group of neurons [68]. This is similar to how a memory engram encodes and reactivates. This encode- and reactivation-like expressing pattern indicates Arc might be involved in neuronal information process and storage.

Studies have shown Arc is required for many forms of cognitive ability and memory. Arc knockout or Arc antisense oligonucleotide-infused mice have impaired long-term memory with the intact ability of forming short-term memory in spatial tasks [57] [58], inhibitory avoidance [69] and fear conditioning [70] [71]. Arc antisense oligonucleotide-infused mice showed impaired long-term memory consolidation [57] in a water maze task. Similarly, Arc knockout mice showed intact learning in water maze trainings, but worsened maintenance of memory of where the spatial goal was [58]. In addition to spatial related memory, contextual fear conditioning was affected by Arc manipulation. With similar behaviors in training sessions, the absent of Arc led animals to significantly less freezing when they were re-exposed to the fear contexts [57] [70] [71]. These studies suggested that Arc is not involved in learning the task but plays a critical role in consolidating the memory. Interestingly, Arc is critical for the reverse learning. In the water maze task, once the platform position was changed, Arc knockout animals spent significantly longer time in previous platform location [58] compared with control animal. It suggested that Arc knockout mice not only have worse memory consolidation, but also less flexibility in reversal learning.

In addition, a recent study showed that persistent Arc expression caused by blocking Arc degradation did not affect spatial learning, but damaged the flexibility in reversal learning [72], indicating that persistent Arc expression blocks acquisition of new information and/or suppression of previous acquired memory.

Arc expression is induced by synaptic and neuronal stimulations. Its activation further regulates many forms of synaptic plasticity, which is critical for memory formation and retrieval. A recent study demonstrated that reactivation of an engram labeled with Arc promoter successfully retrieved memory [73]. This suggests Arc expression correlates with recruitment of neurons into the memory trace. However, further details on how Arc expression changes neuronal activity and contributes to the memory engram have not been answered.

1.2.7 Arc transfers genomic information between neurons

In 2018, a pair of papers showed Arc protein in mice [74] and its homologue in drosophila [75] form a virus-like capsid structure capable of transferring RNA between cells. The C-lobe and N-lobe of Arc form a similar structure to the viral group specific antigen (Gag) protein [27]. Retroviruses such as human immunodeficiency virus use Gag protein to form protective shells which are essential for transport of virus RNA between cells [28]. Similar to Gag, Arc protein encapsulates mRNAs and transfers them between cells, which is a newly discovered mechanism of neuronal communication.

This is the first non-viral protein which has been shown to form virus-like capsids to transfer genome-originated mRNAs between cells. With the report of ~100 other gag-like human proteins [76], additional examples of virus-like cellular communication mechanisms might be unraveled, although understanding of its functional significance needs to wait for future studies.

I.3 Rat hippocampal formation and place cells

The hippocampal formation has been extensively studied ever since its importance in memory encoding was revealed by the findings in patients with hippocampal lesions [7]. The hippocampus is involved in remembering propositional and declarative information [7] [77]. In addition to its critical role in memory, hippocampus also connect to brain regions known to support flexible cognitions and social behavior and contributes to many formats of cognitive abilities, such as decision making, imagination and creativity, social relationship and empathy [78]. There is also evidence that together with anterior cingulate cortex, hippocampus participates in certain form of attention control [79].

Arc expression has been found in hippocampal pyramidal neurons [37]. Some hippocampal pyramidal neurons exhibit location specific firing patterns and are so called place cells [77]. In this study we investigate how Arc activation contributes to place cell activity, and here I will review the anatomical and functional properties of the hippocampus.

I.3.1 Hippocampal anatomy

A well interconnected C shaped bulging structure in medial temporal lobe in the human brain drew attention of early anatomists and got the name 'hippocampus', the Greek word for seahorse, due to its similar shape to this tropical fish (Figure I-4A). People also compared hippocampal structure to the horn of ram and gave it a Latin name cornu

ammonis (CA) [80]. Nowadays, the terminology CA survives to represent hippocampus proper CA1, CA2 and CA3, while hippocampus is a terminology commonly referred to the structures containing the CA regions and the dentate gyrus. Hippocampus exists in each hemisphere and hippocampi in the two hemispheres connect with each other through the ventral hippocampal commissure [81].

The hippocampal formation consists of the hippocampus proper, the subiculum, and fasciola cinereum. The parahippocampal region contains the presubiculum, the parasubiculum, and the entorhinal cortex (Figure I-4B). Another terminology, instead, considers that the hippocampal formation contains the presubiculum, the parasubiculum, the entorhinal cortex, perihinal area and postrhinal area [82]. “Hippocampal region” refers to the hippocampal formation together with the parahippocampal regions (Figure I-4B).

Figure I-4. Hippocampal formation anatomy. **A.** The human hippocampus compared with a seahorse. (Reprint from Wikipedia, Hippocampus_and_seahorse.JPG) **B.** Three-dimensional visualizations of the hippocampal region. Hippocampal regions consists of CA1, cornu ammonis 1; CA2, cornu ammonis 2; CA3, cornu ammonis 3; DG, dentate gyrus; FC, fasciola cinereum; Sub, subiculum; LEC, lateral enthorhinal cortex; MEC, medial enthorhinal cortex; PaS, parasubiculum; PER 35, perirhinal area 35; PER 36, perirhinal area 36; POR, postrhinal cortex; PrS, presubiculum. (Adapted from [82]. Copyright © 2015 The Authors).

In the hippocampus there are two main types of neurons: the principal neurons which project to other subregions and include: pyramidal cells from CA area and granule cells from dentate gyrus. These neurons are excitatory neurons. The other kind is the different types of interneurons which are mainly connected with other neurons in the same area. These neurons can be excitatory or inhibitory and found with various morphologies and functional features [81]. In the CA regions, the cell bodies of pyramidal neurons form pyramidal cell layer or *stratum pyramidale* (Figure I-5). The layer containing the basal dendrites is *stratum oriens*, while *stratum radiatum* and *stratum lacunosum-moleculare* describes the layers containing the proximal and oblique segments of apical dendrites and the layer containing the distal apical dendrite, respectively. CA3 has an additional layer called *Stratum lucidum*, which contains the axon of granule cells in DG, between pyramidal cell layer and *stratum radiatum*. The cell bodies of granule cells in the dentate gyrus form the granule cell layer.

Figure I-5. The layers in the CA1 area. The cresyl violet stained hippocampus shows pyramidal cells in CA area and granule cells in dentate gyrus form dense neuronal layers. In CA1, *stratum oriens* describes the layer containing basal dendrites, while *stratum pyramidale* is an alternative term of pyramidal cell layer and *stratum radiatum* describes the layer containing the proximal and oblique segments of apical dendrites. *Stratum lacunosum-moleculare* is the layer containing distal apical dendrite.

The hippocampal formation is well interlaced, forming a complex circuitry with multiple input/output pathways (Figure I-6). The major cortical input to the hippocampus is from entorhinal cortex layer II and III. Neurons in entorhinal cortex layer II projects to the dentate gyrus, CA3 and CA2, while cells in entorhinal cortex layer III project to CA1 and subiculum complex [83]. The main output projections from hippocampus to entorhinal cortex is from CA2 to entorhinal cortex layer II [84] [85] and the CA1, subiculum complex to cortex layer V/ VI [83]. Inside the hippocampus, the dentate gyrus projects to CA3 through granule cell axons which are called mossy fibers. CA3 in turn connects back to dentate gyrus through pyramidal neurons. CA1 receives input with pathways entorhinal cortex layer III and V/VI → CA1, or entorhinal cortex layer II → dentate gyrus → CA3 → CA1 [86]. Recent years, CA2 area has drawn attention. It receives projections from dentate gyrus and CA3, and projects to CA3 and CA1 [87]. The subiculum receives signals from CA1 [83].

Figure I-6. Hippocampal circuits. CA1, cornu ammonis 1; CA2, cornu ammonis 2; CA3, cornu ammonis 3; DG, dentate gyrus; Sub, subiculum complex; EC, entorhinal cortex. (Copied from [88]. Copyright ©2014 The authors).

I.3.2 Hippocampal electrophysiology

Theta oscillations

The local field potential (LFP) is an electrophysiological signal generated from the electric currents accumulated from nearby neurons [89]. It can be recorded by placing electrodes extracellularly. In freely behaving rodents, several types of oscillations across different frequency bands correspond to certain behaviors [90]. These oscillations are rhythmic waves including theta (6-12 Hz), beta (12-30 Hz), gamma (30-100 Hz), and sharp wave associated ripples (100-200 Hz) [91]. In this thesis work, we have focused on the analyses of neuronal properties related to theta oscillations.

Theta oscillations are large amplitude rhythmical sinusoidal signals (Figure I-7) detected during rapid eye movement (REM) sleep [92] and various types of locomotor activities that can be described as 'voluntary', 'preparatory', 'orienting' and 'exploratory' movements [90]. Theta oscillations are thought to act as an internal clock in the hippocampal circuit by reflecting rhythmical subthreshold membrane potentials [93] and neuronal firing [94] [95]. Theta oscillations can be recorded in all subregions of the hippocampus [96] [97] [98] [99]. The abolishment or manipulations of theta oscillations cause deficits in hippocampus-dependent behavior [100], which indicates the importance of theta oscillations for normal hippocampal function.

Figure I-7. Theta oscillations recorded from the hippocampal CA1 area. Black line: Local field potential. Orange line: Filtered theta oscillations

Single unit activity

Neurons in hippocampus CA area are able to be distinguished in extracellular electrode recordings by waveforms, inter-spike intervals and other properties [101]. First, pyramidal neurons exhibit a longer spike duration than interneurons. Average duration of putative pyramidal cells are around 0.4 msec while putative interneurons have much shorter durations which is around 0.2 msec [102]. Pyramidal cells exhibit complex spikes which contains bursts of 2-6 action potentials with short inter-spike intervals ($\leq 10\text{ms}$) and decreasing amplitude [103]. The average firing rate of principle neurons is generally low, but the rate distribution is irregular such in some short time windows it could reach 20-30 Hz and in others are around 0 Hz. In contrast to this irregular spike time distribution, interneurons exhibit a regular spike pattern across time [102] [104].

1.3.3 Spatially modulated cells

1.3.3.1 Place cells

In 1971, O'Keefe and Dostrovsky [77] found that the firing patterns of some pyramidal cells in the hippocampus was related to the animal's location in an environment. This type of cell fires at a high frequency only when the animal enters a small patch in the environment. The cell is so called a place cell and the area where the cell fires is called a place field (Figure I-8). In a relatively small environments typically used in experiments (e.g. $1 \times 1 \text{ m}^2$), a place cell has one place field or occasionally two. The number of place fields of a single place cell increases with environment size [105]. The size of place fields highly vary, and it increases from the septal to temporal region of hippocampus [106]. Place cells are found in all the subregions in the hippocampus, the CA1-CA3 and dentate gyrus [77] [107] [108] [109]. A small population of place cells were also reported in other hippocampal formation areas [110] [111]. Here I focus on reviewing the place cell properties in the hippocampus, especially those in the CA1 area.

Figure I-8. Place-cell activity of a cell recorded in CA1 region of the hippocampus. Left picture: The gray line shows animal's movement during a 10 min running session in a 1x1 m² arena and red dots show the positions where spikes were recorded from the cell. Right picture: a smoothed color-coded rate map which shows cell's firing rate with colors (Color in the map changes from cold to warm with the increase of firing rate. red: maximum firing rate, dark blue: 0 Hz)

Spatially and temporally modulated firing patterns

A place cell's firing rate progressively increases when animal travels from the edge of its place field to the center of the field, and decreases gradually while it is traveling out of the field [77]. When animal is not in a place cell's field, that cell maintains a low firing rate, with spikes happening at scattered locations [77] [112].

Apart from location specific firing patterns, place cells were found to have a temporal firing pattern with respect to theta oscillations. Spikes from a place cell are locked to a certain phases of the theta oscillations, which means the timing of spikes show more dense distribution around certain theta phases [113]. In addition to theta phase locking, another phenomenon called theta precession is observed. When animal travels through the place field of a given place cell, the first spikes tend to fire at the late phase of the ongoing theta oscillations, and following spikes tend to fire at earlier phases compared with previous spikes. When the place cell exits its place field, spikes are near the beginning of theta cycles [113] [114] (Figure I-9). This phenomenon led to the hypothesis of phase coding, which means the information is encoded by the theta phase that neurons fire at. For example, high-rate place cell firing patterns at the trough of theta are thought to represent the current location, while at the first and second halves show past

and future positions respectively [115]. With the phase coding, theta phase locking and precession of place cells could offer another dimension of coding to increase information that place cell firing pattern represents. In addition to place cells, other spatially modulated cells (described in I.3.3.2) also have temporal specificity in relation to theta oscillations [116]. Given the fact that theta is relatively global oscillations, the firing patterns in regard to the theta oscillation offer a synchronization timer among neurons.

Figure I-9. Phase precession. When animal runs through the place field, the spike theta phase shifts to earlier phase in each theta cycle (separated by gray dashed lines).

Stability and remapping

Many place cells maintain a stable spatial response if the visual stimuli and behavioral context and demands remain stable [112]. Studies have shown that when there is no change in the environment, place cells can have the place fields in the similar area for months [117]. When the animals explore novel environments, place cells can form new stable place fields in the first few minutes, or become silent [118]. This complete change of firing patterns has been termed as 'global remapping' [119]. Apart from global remapping, terms like 'partial remapping' [120] and 'rate remapping' [121] are given to

describe the phenomenon that place cell firing patterns change partially in response to the incomplete environment change (Figure I-10). Partial remapping describes that, in response to partial changes of context, some place cells change place fields or become silent, while other place cells' fields remain at original locations [120]. Rate remapping is when some environment cue is changed, such as the wall color, the location of place fields do not change whereas their firing rate in fields are modified [121].

Figure I-10. Place cell remapping. Top: Two recording environments for each remap category. Bottom: Firing rate maps (dark blue: silent; red: maximum firing rate) of 5 cells in 2 environments in each remapping type. **A.** Place cells fire in one environment become silent or change its place field [118]. **B.** When the environmental change is incomplete (color and order change), some place cells change its firing locations while some remain unchanged [120]. **C.** When environmental context is partially changed, such as color, the location of place fields remain unchanged but the firing rate is modified [121]. (Reprint from [122] Copyright © 2018 Latuske, Kornienko, Kohler and Allen).

Synaptic plasticity is thought to be a cellular basis of formation and stability of place cell activity [123]. By applying high frequency stimulation in rat ventral hippocampal commissure, Dragoi et al. invoked LTP in hippocampus CA1 and CA3 area. This artificially induced LTP altered place cell firing patterns in a familiar environment, similarly to remapping [124]. Genetic and pharmacological methods to block or knockout molecules involved in LTP, such as NMDAR or AMPAR, impairs normal place cell activities including place field formation and maintenance [125] [126] [127] [128-131]. Intracellular recording studies found that dendritic plateau potentials in CA1 pyramidal cells can be triggered by a nonlinear summation of properly timed CA3 and entorhinal cortex [132] [133]. Dendritic plateau potentials are effective at inducing long-term synaptic plasticity [132], generating complex burst firings [134] [135] [136], as well as forming new place fields [133]; but also see Cohen et al. [137],. These studies together indicate that LTP-like synaptic plasticity is critical for place cell activity. Synaptic strengthening seems to support the establishment of new place fields, and the long-lasting synaptic strengthening maintains a stable firing pattern of place cell in a familiar environment.

I.3.3.2 Other spatially modulated cells

Many other spatially modulated cells were discovered in parahippocampal regions and considered as elements in spatial signal processing. Here I will give a brief description of them based on the sequence that they were reported.

Figure I-11. Spatial modulated cell types. **A.** Head direction cells. An example of head direction cell tuning curves. (Adapted from [138]. Copyright © 1997 Elsevier Science Ltd.). **B.** Grid cells. Left: an example of a grid cell recorded in a 2.2x2.2m² square box. The gray line shows the trace of animal's movement and black dots show the positions where spikes were recorded from the cell. Right: Schematic of grid cell firing pattern. Blue dots are place fields of a grid cell. They form hexagonal pattern (black lines) sharing similar orientation (dashed lines) and scale or wavelength (solid lines with arrows). (Adapted from [139]. Copyright © 2016 by Annual Reviews.) **C.** Boundary vector cells. Two rate maps examples of a boundary vector cell (dark blue: silent; red: maximum firing rate). (Adapted from [140]. Copyright © 2009 Society for Neuroscience). **D.** Rate maps of a border cell (dark blue: silent; red: maximum firing rate). Left: Border cell has firing field along the wall. Right: Adding a new border into the same environment causes a new border field from the same border cell. (Adapted from [141]. Copyright © 2008, American Association for the Advancement of Science).

Head direction cell

Firing of head direction cells represent the direction of the animal's head by showing high firing rate when the head is facing its preferred direction (Figure I-11A) [142] [143] regardless of animals' position. The range of the directions one head direction cell could represent varies from $\sim 60^\circ$ to $\sim 150^\circ$. Head direction cells were discovered in many brain regions, including the postsubiculum [142], the lateral dorsal thalamus [144], the dorsal striatum [145], the anterior dorsal thalamic nucleus [146], the lateral mammillary nuclei [147], and the retrosplenial cortex [148], as well as the entorhinal cortex [149]. One hypothesis to explain why head direction cells exist in many brain regions is that the directional signal is important for many different cognitive functions corresponding to different brain regions. Existence of head direction cells in the brain region required for the cognitive task could directly offer direction signals the task requires.

Grid cell

Grid cells are cells in the entorhinal cortex which have multiple discrete place fields and these fields form a hexagonal grid pattern repeating over the entire space in a given environment [150] (Figure I-11B). Neighboring grid cell share similar properties, which include grid orientation, the angle of grid patterns; and grid scale, the distance of two adjacent fields. Grid scale increases from dorsal to ventral medial entorhinal cortex, suggesting that they can represent space in different scales [151] [150] [152]. Grid cells' representations of the whole environment with a regularly repeated pattern could provide animals with a sense of distance. The different scales of grid pattern could offer

different resolution of distance. Thus grid cells may work as metric for space in animals [139].

Boundary vector cell and border cells

Two cell types exhibiting firing patterns related to boundaries, border cells and boundary vector cells, were discovered around a similar time. Boundary vector cells' existence were predicted by multiple modeling studies [153] [154] and were experimentally identified in the mEC and subiculum [140]. The receptive field of boundary vector cells is along the boundary and firing rate inversely increases with the distance of the animal to the boundary, regardless of animals' head directions (Figure I-11C). Border cells exist in mEC and adjacent parasubiculum [141]. The difference from boundary vector cells has narrow firing field directly adjacent to the boundary, probably encoding border itself only (Figure I-11D), whereas boundary vector cells have a larger receptive field which could represent animals' distances to the wall. If new borders are added or original borders are moved to other positions, boundary vector cells and border cells will form fields along the new boundaries as long as these boundaries are aligned in the same directions as the wall along which the original field was located.

I.3.3.3 Spatial inputs to CA1 place cells

CA1 pyramidal neurons receive synaptic input from the third layer of medial entorhinal cortex to their tuft dendrites. CA2 pyramidal neurons project to the stratum oriens of CA1 pyramidal neurons, while CA3 projects to stratum radiatum of CA1 pyramidal neurons. All these three regions contain spatial modulated neurons (entorhinal cortex: grid cells, head direction cells, boundary cells etc; CA2 and CA3: place cells) [86] [87]. They are thought to be the upstream and give spatial input to CA1 place cells. Multiple different models tried to unravel how grid cell contribute to form a place field in CA1 place cell. Experimental studies showed inactivation of medial entorhinal cortex leads to an expansion of place field size in CA1 [155], as well as unstable spatial representation [156]. These studies also showed without entorhinal input, CA1 place cells maintain their spatial representation ability, which could be contributed by CA2 and CA3 or even other sensory inputs.

Overall, CA1 place cells converges spatial signals from CA2, CA3 and entorhinal cortex. Information processed upon different times may be from different inputs and form different circuit. The clear function of how each input contributes to CA1 place cell requires more investigation.

I.4 Aim of the study

Arc is suggested to be an important mediator of activity-dependent changes in the brain and is used as biomarkers to label memory engram. It bridges experience with synaptic modification and is critical for long-term memory. Despite the accumulating findings that Arc regulates synaptic plasticity and memory, how Arc contributes to the generation of neuronal firing pattern is not clear. In this study, we aim to understand what aspects of behaviorally-relevant neuronal activity is regulated by Arc. For this purpose, we used place cells as a model, and examined how interfering the expression of Arc gene affects place cell activity in freely moving rats.

II Methods

II.1 Screening of shRNA sequences against Arc (performed by Ingrid Åmellem and Chika Yoshii)

Four pre-miRNA sequences against Arc gene and one control sequence designed by Invitrogen (Arc 92, Arc 93, Arc 94, Arc 95) were cloned into pcDNA™6.2-GW/EmGFP-miR expression vector (BLOCK-iT™ Pol II miR RNAi Expression Vector Kits, Invitrogen). The resulting plasmids produces miRNAs under the control of cytomegalovirus promoter. These miRNAs target the following sequences:

Arc 92: GAAGAACTGGGTGGAGTTCAA

Arc 93: TGACAGGACCCAGCCTGAATA

Arc 94: AAGCTGATGGCTACGACTACA

Arc 95: CAAACCCAATGTGATCCTGCA

Control: AAATCGCTGATTTGTGTAGTC

In vitro knockdown efficiency was evaluated by co-transfecting a plasmid for expressing mCherry-fused Arc gene under the control of the cytomegalovirus promoter and one of five miRNA plasmids described above into AAV293 cells (Stratagene). If Arc gene expression is affected by miRNAs, the expression of mCherry, which is fused to Arc, is also affected. In vitro knockdown efficiency was quantified by calculating reduction in the fluorescence intensity of mCherry compared with control. Larger reduction corresponds to better knockdown efficiency.

II.2 Recombinant adeno-associated viral vectors (Prepared by Mehdi Fallahnezhad)

We constructed recombinant adeno-associated viral (rAAV) transfer plasmids containing inverted terminal repeat sequence (ITR), the Ca^{2+} / calmodulin-dependent protein kinase II alpha (CaMKII α) promoter, the coding sequence of Emerald green fluorescent protein (EmGFP), four stretches of one of three miRNA sequences (Arc 92 Arc 95 or control), the Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE) and human growth hormone poly A as shown in Figure II-1.

Figure II-1. Structure of recombinant adeno-associated viral vectors containing miRNA sequences against Arc gene or control miRNA sequence.

Serotype 1/2 rAAV vectors were produced by co-transfecting one of three AAV transfer plasmids described above with three helper plasmids, pAAV-RC, pHelper (Stratagene) and pXR1 [157] to AAV293 cells (Stratagene) [158] [159]. AAV293 cells were cultured in a humidified 37°C incubator which contains 5% CO_2 . The culture medium contained Dulbecco's Modified Eagle Medium (DMEM) (Gibco® Life Technologies), sodium pyruvate (1% volume of DMEM) (Gibco® Life Technologies), MEM Non-Essential Amino Acids (1% volume of DMEM) (Gibco® Life Technologies), Fetal Bovine Serum (10% volume of

DMEM) (Gibco® Life Technologies) and Penicillin-Streptomycin (1% volume of DMEM) (Gibco® Life Technologies).

One day before transfection, cells were seeded in 5 15-cm dishes in 25 ml culture medium without Penicillin-Streptomycin. Four plasmids including the plasmid of interest (22.5 µg/plate), the pHelper plasmid (22.5 µg/plate), the pXR1 plasmid (11.3 µg/plate) and the pAAV-RC plasmid ((11.3 µg/plate), were mixed with 0.333 M CaCl₂ (2.25 ml/plate). The mixture was filtered with 0.2 µm filter (Pall Corporation, Acrodisc 13 mm Syringe Filter), and then mixed with 2x Hepes buffered saline (2.25 ml/plate) [160]. 4.5 ml of the mixture was added to each 15-cm plate for transfection. Six hours after transfection, the medium was replaced with 20ml of fresh culture medium containing Penicillin Streptomycin.

Forty-eight hours after transfection, AAV cells together with the medium were transferred to two 50-ml tubes. AAV293 cells were spun down by centrifugation at 200g for 10 min. Mediums were aspirated after centrifugation and 10 ml Dulbecco's phosphate-buffered saline was added to each tube for another centrifugation (200g, 10 min). Cells were re-suspended with 25 ml of 150 mM NaCl, 20 mM Tris-HCl (pH 8.0) per tube. 10% sodium deoxycholate were added to reach a final concentration of 0.5%, together with benzonase nuclease at final concentration of 50U/ml. The tubes were then incubated in a 37°C water bath for 60 min before centrifugation at 3000g for 15 min. The supernatant was heated in 56°C water bath for 15 min and then frozen in a dry ice/70% ethanol bath. After thaw the frozen solution in a 37°C water bath, centrifugation at

3000g for 15 min was applied to remove cell debris. The supernatant was filtered with a 0.45 μ m pore filter (32-mm 0.45- μ m Acrodisc syringe filter, pall).

Purification and concentration of rAAV vector was performed by using heparin affinity columns (1 ml HiTrap Heparin HP, GE Healthcare). Heparin column was pre-equilibrated with 10ml of 150 mM NaCl, 20 mM Tris-HCl (pH 8.0). After the supernatant was passed through the column, rAAV vectors were eluted with solutions as follows: 1 mL 300 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1 mL 400 mM NaCl, 20 mM Tris-HCl (pH 8.0), 2 mL 450 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1 mL 500 mM NaCl, 20 mM Tris-HCl (pH 8.0). 5 ml of the eluted solution were transferred into centrifugal filter devices (Amicon Ultra-4, 100 K normal molecular weight limit, Millipore, Billerica, MA) and centrifugated at 3000g to reach a final volume of <500 μ l. The rAAV were aliquoted and stored at -80°C.

II.3 Evaluation of Arc knock down efficiency in vivo (Done by Yao Mei Wang, Ching Ching Chang, Li Yuan and Ayumu Tashiro)

Knockdown efficiency in the AAV-transduced hippocampal CA1 area was examined using reverse transcription quantitative polymerase chain reaction (RT-qPCR) (Figure II-2). Rats were injected with the viral vector containing miRNA against Arc (Arc 92 or Arc 95) into one hemisphere (injection method is described in II.5), and the control viral vector into the other hemisphere. More than thirty days after virus injection, rats were exposed for 5 min to a 1m x 1m box which they had never explored, to activate Arc transcription. Immediately after exploration, rats were placed into a cage covered by a black plastic bag and transferred to a procedure room. Ten min after the end of exploration, rats were anesthetized with isoflurane and euthanized. Brains were extracted and sectioned in 200µm thickness using vibratome (VT-1200S; Leica, Germany) with artificial cerebrospinal fluid (ACSF; in mM: 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 25 dextrose, 2 CaCl₂, 2 MgCl₂, 3 Na-pyruvate, 1 ascorbic acid, maintained at pH 7.4 by gassing with 95% O₂ / 5% CO₂) . Each brain slice containing the hippocampus were observed with fluorescent microscope. EmGFP positive portions of CA1 area were dissected separately from each hemisphere and total RNA was extracted with RNA isolation kit (QIAGEN). cDNA was synthesized with reverse transcription (The GoScript™ Reverse Transcription System, Promega) and used as template DNA for qPCR using Arc specific sequences (Arc forward: CCCTGCAGCCCAAGTTCAAG, Arc reverse: GAAGGCTCAGCTGCCTGCTC) as primers.

Figure II-2. In vivo Arc knockdown efficiency check. Thirty days after virus injection, rats were put in a novel 1m x 1m box to perform spatial exploration for 5 min. 10 min after the end of exploration, rats were anesthetized and euthanized before termination. Brains were extracted and sectioned with 200μm thickness. Virus transduced hippocampal CA1 areas (green area) were dissected and collected under a fluorescent microscope. Total RNA was extracted and RT-qPCR analysis was performed.

Four animals were used for comparison between Arc 92 and Ctr, and 2 animals were used for the comparison between Arc 95 and Ctr. For each individual animal, Arc RNA amount in Ctr and Arc knockdown hemispheres were normalized by the Ctr hemisphere of this animal. In this case, Arc expression bias from animals and behaviors are limited. With this normalization, RNA amount from Ctr hemisphere is always 100%.

II.4 Subjects and behavioral task

Subjects were 6 to 10-week-old Long-Evans rats at the time of training started. They were housed individually in transparent cages in a room which was under 12h light/dark cycle. Rats were trained to forage food (Milo cereal, Nestle) scattered on the floor in a square open field (dimensions 100 cm × 100 cm; walls with 50 cm height) in a room before surgery (describe in the next section) for at least 10 days. Training in the same box in the same room restarted one week after surgery, while electrophysiological data was collected. Rats were trained to forage the food scattered on the floor in the box for 10 min. Five minutes rest was allowed between each 10 min exploration. Training sessions vary between 3 to 6 sessions a day. When recording tetrodes were estimated to be near the area of interest (detailed description in II-7), 6 sessions were performed in a day (Figure II-3). To motivate them to collect food, the rats were food restricted. Once in 1-2 weeks, the rats were given ad libitum access to food and the amount of food a rat per day was measured. Seventy percent of the free-eating food amount was given to that rat every day before training.

Figure II-3. Time line of the food foraging task. Rats were allowed to explore the open box A for 10 min with 5 min rest between sessions.

II.5 Surgery

Rats were deeply anesthetized with isoflurane (4% in oxygen, 2 L/min flow).

Buprenorphine (0.3mg/kg body weight) was injected as an analgesic. Using a stainless burr (FST, cat. no. 18004-23), two holes with the diameter of 2.3 mm were drilled on the skull, with the coordinate of 4.0 mm posterior to the bregma, 2.4 mm lateral to the midline (left and right; Figure II-5, yellow holes).

0.5 μ L of viral vector solution was injected into the dorsal CA1 area using a micro-syringe (800 RN, Hamilton), with the coordinate of 4.0 mm posterior to the bregma, 2.4 mm lateral to the midline (left and right), 2.5 mm ventral from the brain surface as shown in Figure II-5. For the rats used for electrophysiological recording, 4 recording tetrodes mounted on a microdrive (Axona, cat.no.MDR16) were implanted in each hemisphere from the same hole as for virus injection with the coordinate of 3.8 mm posterior to the bregma, 3.1 mm lateral to the midline and 1.5 mm ventral to the brain surface (Figure II-4). Tetrodes and microdrives were implanted with 10° tilt towards a lateral side. Eight stainless screws (TR Fastening, cat. No. 70416003; Figure II-5, black and blue dots) were implanted on the skull to assist the stable attachment of microdrives together with dental cement. Among the eight screws, three (Figure II-5, black dots) were used as ground screws by connecting to the ground channels of microdrives or recording system.

Arc knockdown:

AAV1/2-CK1-EmGFP-miR-Arc95x4

AAV1/2-CK1-EmGFP-miR-Arc92x4

Control:

AAV1/2-CK1-EmGFP-miR-LacZx4

Figure II-4. Bilateral injection and microdrives implantation. Rats were injected with the viral vector containing shRNA miRNA against Arc (Arc 92 or Arc 95) into one hemisphere (injection method is described in II.5), and the control viral vector into the other hemisphere. Then tetrodes mounted on a microdrive were implanted in each hemisphere.

Figure II-5. Schematic of screw and craniotomy locations. Blue marks: screw positions for attaching drives to the skull. Black marks: screws used for grounding headstages. Yellow circle: holes for virus injection and tetrode insertion.

II.6 Microdrive and recording hardware setting

Tetrodes used for unit recording were made by twisting heavy polyimide-coated 90% platinum-10% iridium wires with the diameter of 17 μm (California Fine Wire, Grover Beach, CA) into a bundle. Microdrives carrying 4 tetrodes were mounted on skull for a stable chronic recording (Figure II-6). One day before we implanted microdrives into rat brains, tetrode tips were electroplated in platinum plating solution so that the impedance were reduced to 200-300k Ω measured in 0.9% NaCl solution with 1kHz.

Figure II-6. Microdrive. Left: Schematic of microdrive design. Right: Microdrive before implantation. Pictures are from Axona website.

For unit recording, signals were amplified 5000-10000 times and filtered with a 600-6000 Hz bandpass using DacqUSB digital data acquisition system (Axona Ltd, UK). Spike timestamps and waveforms were saved when the signal passed a threshold (adjusted in

the range of 50-60 μV). For local field potential (LFP), signals were amplified 1000-2500 times and filtered with a low pass filter whose cut-off frequency was 500 Hz.

Rat locations were monitored by tracking the LED lights on the headstages, acquired through an overhead-mounted camera (Figure II-7).

Figure II-7. Data acquisition. The Axona system was connected to microdrives to record electrophysiological data while animal was exploring the arena. Animal position was recorded through a camera by the recording system. Synchronized positions, spikes and LFP data were saved for offline analysis.

II.7 Tetraode advancing and recording site estimation

Recording sites were estimated from recording results, and when necessary, tetrodes were advanced by 50 μm deeper into the brain after last recording sessions of the day. The position of recording site was estimated from the LFP signal. From the cortex to hippocampal CA1 layer, theta oscillation (5 -10 Hz) appear up in the LFP while the animal is exploring the environment (Figure II-8), and becomes stronger when the recording site becomes closer to the CA1 pyramidal cell layer.

Figure II-8. A screen shot taken from an oscilloscope in the recording system displaying signals over time. The screen shot was taken while animal was moving. Clear theta oscillations showed up in each channel indicated by A & B, which show low pass filtered (0Hz-500 Hz) signals used for local field potential (LFP) recording. Each row corresponds to a different tetraode. The whole horizontal axis is equal to 2 seconds. Section A shows LFP from left hemisphere. Section B shows LFP from right hemisphere.

When the tetrode tip is in the CA1 pyramidal cell layer, intermittent sharp waves (SPW; 40-120 msec duration), and SPW-associated high frequency oscillations (ripples; 150-200 Hz) are observed during behavioral immobility and sleep. Along the depth in the CA1 area, sharp wave shape changes gradually and inverted at stratum pyramidale; ripples happen near SPW peak in stratum oriens and stratum pyramidale, while in stratum radiatum, ripples happen near SPW trough [161]. Figure II-9 shows a screen shot taken from the oscilloscope in the recording system when the rat was not moving. This figure shows the occurrence of high frequency ripples associated with SPW, which indicates the recording site was around the stratum oriens. Based on these criteria, we estimated the position of recording sites relative to the stratum pyramidale. Once the recording site was estimated to be in the stratum pyramidale and many place cells were recorded, we stopped lowering the tetrode to maintain stable recording for final recording sessions.

Figure II-9. Sharp wave ripples. A screen shot taken from the oscilloscope in the recording system displaying LFP recording while animal was immobile. A & B: Low pass filter (0 Hz-500 Hz) channels used for LFP recording. Each row corresponds to a different tetrode. A shows LFP from left hemisphere. B shows LFP from right hemisphere. C & D: Band pass filtered (60-300 Hz) signal from a channel from a tetrode used for LFP recording. By removing low frequency LFP events, these signals visualize high frequency ripples. C is one channel from the left hemisphere while D is from the right hemisphere.

II.8 Histology

After we finished all the recording, animals were perfused with saline and then 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were sectioned coronally with the thickness of 40 μ m for checking the virus transduction and tetrode track. Sections were mounted on slides using polyvinyl alcohol mounting medium with 1,4-diazabicyclo[2.2.2]octane and coverslips. Fluorescence images were taken with an Axio Scope A1 microscope (Zeiss, Oberkochen, Germany). Viral transduction in the CA1 was confirmed by observing the distribution of EmGFP fluorescence. After the fluorescence imaging, the coverslip was removed and the sections were stained with cresyl violet. Cresyl violet was used to demonstrate the Nissl substance in the neuronal and cell nuclei. By applying the staining the trace caused by tetrodes becomes clearer on the brain sections. Transmitted light images were taken with the same microscope and from the same regions as fluorescence images. By examining EmGFP fluorescence from infected area and track of tetrodes, we confirmed that recording was performed from virus transduced area.

II.9 Data analysis and statistics

II.9.1 Spike sorting and unit classification

Spike sorting was done manually with the software Tint (Axona Ltd, St. Albans, UK). In Tint, spikes were sorted based on the amplitude and waveforms recorded on different channels. We sorted the spikes into different clusters (Figure II-10). The clusters were classified as principal cells (putatively recorded from pyramidal cells) if they met the following criteria [126]: (1) In histogram of inter-spike intervals, there is no or few inter-spike intervals within 0-2 ms range, followed by a sharp peak of short inter-spike intervals within 2-10 ms. (2) The average firing rate is (the number of spikes divided by the session duration) between 0.1 Hz to 5Hz. (3) Mean spike width (spike waveform peak to trough time length) is greater than 0.2 msec. (4) Isolation distance (measurement of cluster separation from other spikes) [162] is not less than 10.

Figure II-10. Spike sorting and classification into principal cells. A. Cluster separation by different amplitude and other parameters. Each point represents the peak-trough amplitude on one electrode against the peak-trough amplitude on another. Because a tetrode has four electrodes, totally 6 projections are shown. **B.** Histogram of inter-spike intervals. Principal cells show no inter-spike intervals within 0-2 ms, followed by a sharp peak at 2-10 ms.

II.9.2 Map generation

After spike sorting and putative pyramidal cells identification, rate maps were generated by a custom-written program in the Matlab software. A rate map shows the cells firing frequency in different positions of the environment. The $1 \times 1 \text{ m}^2$ large environment was divided into $5 \times 5 \text{ cm}^2$ spatial bins. The firing rate was calculated in each bin as the number of spikes divided by the time spent in the spatial bin. Both spike number and time were smoothed by a Gaussian kernel. A color code was then used to represent different firing rates in all positions in a way that color becomes warm when the rate is high (Figure II-11).

Figure II-11. Representation of path and corresponding rate map. **A.** Animal trajectory and spike location. The gray line shows the trace of animal's movement during a 10 min foraging session in a 1m x 1m arena and red dots show the positions where spikes were recorded from the cell. **B.** A smoothed color-coded rate map which shows cell's firing rate with colors in behaving environment (Color in the map changes from cold to warm with the increase of firing rate. red: maximum firing rate, blue: 0 Hz).

II.9.3 Single unit analysis

The following analysis for individual cells were calculated and compared between groups. If not specified, independent T test were applied to test differences for control vs Arc 92 and control vs Arc 95, for following analysis.

Average rate:

The average firing rate of each cell for a session was calculated by dividing the number of spikes during the session by the session duration.

Peak rate:

The peak rate of each putative pyramidal cell was defined as the highest firing rate observed among spatial bins of the rate map in a recording session.

Single spikes and burst event:

Single spikes are defined as spikes separated from other spikes with more than 6-ms inter-spike interval. Burst event is a group of two or more consecutive spikes with not more than 6-ms inter-spike intervals between consecutive spikes.

Place field detection:

Detection of place fields started from highest rate bin in the whole map. When the highest rate bin ≥ 1 Hz and there were ≥ 8 neighboring bins with at $\geq 20\%$ of peak rate, those bins were considered as a place field. Once a field was detected, areas inside that

field are removed from next field detection. If no place field was detected with the current highest rate bin, the highest rate bin was removed from next field detection. And the next detection of a place field started from highest rate bin of remaining bins. This procedure was repeated until there are no qualified fields. The first detected field was considered as the main place field of a cell.

Average rate inside and outside place field:

After the place field is determined with the criteria described above, spikes inside and outside place field were separated and corresponding time animals stayed inside and outside field were counted. The average rate inside and outside field were calculated by dividing the numbers of spikes by time. No Gaussian smoothing was applied in this analysis.

Spatial contrast:

Spatial contrast describes how different the inside and outside field average firing rates are. By comparing out- to in-field rate, the higher ratio it is, the lower spatial contrast the place cell has:

$$\text{Out} / \text{In-field rate}$$

Map correlation:

The spatial pattern similarities of one neuron between sessions were quantified by comparing the rate maps between 2 consecutive sessions. Pearson correlation coefficient was calculated for corresponding smoothed spatial bins of two sessions. If

one bin is unvisited in any session, value of this bin was removed from both sessions.

There were 6 sessions in one day, and thus coefficients were calculated for 5 consecutive session pairs. The average of the 5 correlation values was used to represent the spatial correlation of that neuron.

Coherence:

Spatial coherence is a measure of the “smoothness” of spatial firing patterns. It is calculated as the correlation coefficient of the firing rate in each pixel with the averaged firing rate from surrounding 8 pixels [163].

Information (bits per second & bits per spike):

Spatial information is calculated based on Shannon’s information theory to quantify the information the cell carries in the session. The amount of information per second is calculated as following:

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

where p_i is the probability density for the rat to be in location bin i , λ_i is the mean firing rate when the rat is in the location bin i , λ is the overall mean firing rate of the cell [164].

The amount of information per spike is calculated as Information bits per second divided by mean rate. With the formula for information (bits per second), if we divide it with the overall mean firing rate λ , then the result becomes bits per spike as the unit for λ is spikes/second.

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

Sparseness:

Sparseness is a measure of how diffuse the firing pattern is in the environment [165].

The larger the value of sparseness, the more diffuse the place field is.

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

Velocity threshold:

A velocity threshold of 5 cm/s was applied to remove the variances generated from animal behaviors. Data acquired while animal's speed is over 5 cm/s were kept for analysis. Average rate, peak rate, field number, field size (main and sum), burst rate, single spike rate, in field rate, out field rate and spatial contrast were analyzed with the velocity threshold.

II.9.4 LFP properties**Theta oscillation property**

LFP data was recorded while animal performed behavioral tasks. Theta oscillation was extracted by applying a bandpass filter (5-12 Hz). Theta phase was assigned by using Hilbert transform (Figure II-12A). To check whether basic properties of these global oscillations were changed by local Arc knockdown, theta frequency, power, and shape were compared between knockdown and control hemispheres.

Theta frequency was calculated as theta wave peaks (highest amplitude value) per second.

$$Theta\ frequency = \frac{Theta\ peak\ numbers}{Session\ length}$$

Theta power was proportional to the normalized summation of amplitude (voltage) square.

$$Theta\ power = \frac{\sum_{n=1}^m Theta\ amplitude^2}{m}$$

where m is the number of amplitude samples.

Theta waveform is sinusoids shape. To check if the sinusoid shape was changed by Arc knockdown, each theta oscillation cycle was divided in 30 degree bins and time spent in each bin was quantified and compared between groups (Figure II-12B).

Figure II-12. Theta filtering, phase assignment and phase bin division. A. Theta extraction and phase assignment. Black: Raw LFP. Orange: Filtered theta oscillations. Green: Theta phase assigned by Hilbert transform. B. One theta cycle is divided into 12 phase bins to check if theta shapes were same in different groups.

Spike theta phase locking

To investigate spike timing on theta oscillations, theta phases at the time of spike firing were assigned to individual spikes as the theta phases of the spikes (Figure II-13A).

Histogram of spike theta phases were generated to help visualizing the spike distribution along theta oscillations (Figure II-13B).

Figure II-13. Spike phase assignment and histogram.

A quantified value, pairwise phase consistency [166], was used to evaluate how concentrated these spikes are on theta phases. Pairwise phase consistency [166] is an unbiased quantification method by calculating average angular differences of circular data sample pairs.

$$PPC = \frac{2}{N(N-1)} \sum_{j=1}^{N-1} \sum_{k=j+1}^N f(\theta_j, \theta_k),$$

where N is the total spike number, θ_j, θ_k are phases from j th and k th spike. Function f calculates angular differences:

$$f(\varphi, \omega) = \cos(\varphi - \omega) = \cos(\varphi) \cos(\omega) + \sin(\varphi) \sin(\omega).$$

Value of function f is reversely correlated with the phase differences between two spikes. Thus a high pairwise phase consistency value means that spikes phases are close to each other.

II.9.5 Population analysis

Cofiring

Pairs of neurons from the same hemispheres were analyzed for how often they cofired. In a neuron pair of neuron 1 and neuron 2, given a spike in neuron 1 occurred at time T , numbers of spikes from neuron 2 happening within the time range $T \pm 100$ ms was considered as the number of cofire counts of the spike in neuron 1 (Figure II-14). This number was summed for all the spikes from neuron 1 to calculate the total cofire events in this pair of neurons.

Figure II-14. Cofire diagram.

Decoding

The arena was divided into 400 (5 cm x 5 cm) spatial bins. For a given spatial bin i , firing rates of neurons were calculated by randomly picking 500 ms of unit recording data from each neurons while the rats were inside the bin. If a viral vector group (control, Arc 92 or Arc 95) contains N neurons, we can get an $N \times 1$ firing rate vector for spatial bin i . The average firing rate vector was calculated for each neuron at each position, forming an $N \times 400$ average firing rate matrix. Correlation coefficient values were calculated between

the $N \times 1$ firing rate vector of position i and each individual column of $N \times 400$ average firing rate matrix of all positions (Figure II-15). The position with highest correlation coefficient was treated as decoded position. Its distance to real position i was named as decoding error distance. The average error distances were calculated from each estimation with different real Position i . This procedure was repeated for 1000 times.

Correlation analysis is sample numbers, which is the cell number N here. The cell numbers were 78 in control group, 51 in Arc 92 group and 54 in Arc 95 group. To avoid the bias from sample numbers, sub sampling analysis that uses 100 cell sets from combination C_{78}^{51} from control and 100 cell sets from combination C_{54}^{51} were performed. For each cell set, decoding analysis was done in the same way as described above.

Figure II-15. Decoding diagram. Firing rates for each neuron at position i were calculated by considering random continuous 500 ms of that neuron staying in the position i . Hence an $N \times 1$ firing rate vector was generated. Correlations were calculated between $N \times 1$ firing rate vector from position i and each individual column of $N \times 400$ rate matrix. The position with highest value was the decoded position.

III Results

III.1 Arc knockdown efficiency, in vitro and in vivo

Knockdown efficiency of four miRNAs against Arc were tested in vitro. Plasmids encoding mCherry fused Arc were cotransfected with plasmids encoding either one of miRNAs against Arc or control miRNA (LacZ) into AAV 293 cells. Knockdown efficiency was revealed by the reduction of fluorescence intensity of mCherry. Compared with control, fluorescence intensity decreased in preparation with all 4 miRNAs against Arc. Among them, Arc 92 and Arc 95 showed the most successful knockdown of Arc gene (Figure III-1). Thus, viral vectors containing Arc 92 or Arc 95 were made.

Figure III-1. Arc gene knockdown, in vitro. A1-5: mCherry fluorescence images of transfected cells. Cotransfection of plasmids encoding Arc-mCherry with plasmids encoding one of four miRNAs against Arc gene or a control miRNA in AAV 293 cells. B: Quantification of mCherry fluorescence intensity from 5 transaction of each plasmid. Preparations with Arc 92 and Arc 95 showed the lowest intensities. * P ≤ 0.05.

In vivo knockdown efficiency was examined by analyzing Arc mRNA amount in AAV vector transduced area, which is indicated by the co-expressed EmGFP in the viral vectors. Using RT-qPCR, we found Arc 92 and 95 reduced Arc mRNA by 19.98% and 51.38%, respectively in EmGFP expressing parts of CA1 area (Figure III-2B).

Figure III-2: Localized and highly efficient viral transduction in the hippocampus CA1 area. **A.** Tile scanning (5 x 9 field of views) confocal microscope images showed rAAV mediated EmGFP transduction in CA1 area. Green: EmGFP; Scale bar, 500 μ m. **B.** RT-qPCR analysis of Arc mRNA expression in the virus-transduced CA1 area. Values are presented as the relative quantification of Arc expression compared with control group.

III.2 Localized high-efficient virus transduction

Pharmacological or transgenic knockdown or knockout method manipulating Arc or other molecules involved in synaptic plasticity caused oscillatory [167], cognitive [58] and behavioral changes [168]. Impairment of place cell firing patterns are associated

with these changes [125] [169] [129]. A global manipulation makes it unclear if the observed neuronal firing changes are directly from cellular interference or indirectly from systematic changes. To minimize such ambiguity, we injected viral vector in a small portion of CA1. A histological analysis was done to examine whether the injection protocol can achieve localized transduction. The results showed a small portion of CA1 area expressed EmGFP in rats injected with each of three viral vectors (Figure III-3, Table III-1).

Figure III-3: Localized virus transduction. Sections were prepared 1-2 months after injection. Images of hippocampal CA1 area injected with a rAAV expressing EmGFP and miRNAs showed localized expression of EmGFP. Scale bar, 1mm. A. Coronal sections containing injection sites are shown for each of the viral vector group. B. Coronal sections containing an injection site (middle) and -760 μm anterior (left) and 760 μm posterior (right).

For each hemisphere that we used in data analysis, from anterior to posterior direction, first slide contains clear fluorescent signal was used to estimate the anterior boundary of virus expression. The section contains needle track (if no needle track was found, then use the most bright section checked by eye) was the injection site section. The last section contains clear fluorescent signals was used as the posterior boundary. The expression boundary is listed in the table below as anterior and posterior coordinates. Distances of these sections from bregma was estimated by comparing the sections with 'The Rat Brain in Stereotaxic Coordinates' [170]. Bregma was used as origin point 0 and sections coordination was estimated through anterior-posterior axis (AP).

Animal	Hemisphere	Virus	AP		
			Anterior	Injection site	Posterior
139	Left	Arc 95	-2.76 mm	-3.48 mm	-4.32 mm
139	Right	LacZ	-2.52 mm	-3.80 mm	-4.28 mm
412	Left	Arc 92	-2.88 mm	-3.72 mm	-4.28 mm
497	Right	LacZ	-3.72 mm	-4.52 mm	-5.00 mm
505	Left	LacZ	-2.72 mm	-3.48 mm	-4.28 mm
505	Right	Arc 92	-2.76 mm	-3.60 mm	-4.48 mm
556	Left	Arc 92	-3.44 mm	-3.48 mm	-3.96 mm
556	Right	LacZ	-3.08 mm	-3.12 mm	-4.08 mm
596	Right	Arc 92	-3.16 mm	-3.96 mm	-4.68 mm
617	Right	Arc 95	-2.92mm	-3.48 mm	-3.76 mm
684	Left	Arc 95	-3.40 mm	-4.20 mm	-4.48 mm
684	Right	LacZ	-3.48 mm	-3.72 mm	-4.56 mm
725	Left	LacZ	-2.72 mm	-3.48 mm	-3.96 mm
725	Right	Arc 95	-3.00 mm	-3.40 mm	-3.76 mm
744	Left	LacZ	-3.00 mm	-3.16 mm	-4.00 mm
745	Left	LacZ	-3.24 mm	-3.28 mm	-3.36 mm
786	Left	Arc 95	-3.12 mm	-4.36 mm	-4.76 mm
786	Right	LacZ	-3.24 mm	-3.80 mm	-4.84 mm
798	Right	LacZ	-2.68 mm	-3.56 mm	-4.20 mm

Table III-1. Anterior-posterior spread of EmGFP expression estimation. The estimated coordinates (from the bregma) of the most anterior and posterior sections with EmGFP expression are listed.

To quantify the efficiency of the virus transduction, virus injected brain section were stained with DAPI to visualize the nuclei (Figure III-4A). Around center area of injection, 89% of neurons in control group were GFP positive and 85% in Arc 95 group out of all DAPI positive neurons counted (Figure III-4B). DAPI also labeled interneurons. This means in pyramidal neurons in that region, transduction efficacy was higher than those numbers.

Figure III-4. Transduction efficiency. **A.** Sections were prepared one month after injection. Efficient transduction (green) in dorsal CA1 pyramidal cells near injection site. Scale bar, 50μm. **B.** Transduction efficiency of AAV vectors. The figure showed GFP positive neurons out of all neurons in a spot chosen around the center of injection. Control and Arc 95 virus injections were performed on different hemisphere in one animal. For each hemisphere, 2 sections near injection site were used for counting DAPI and GFP positive neurons. In control, there are 302 GFP+ neurons out of 338 neurons. In Arc 95 group, there are 321 GFP+ neurons out of 364 neurons.

III.3 Tetrode track and unit classification

To verify recorded cells were from the middle of virus-transduced CA1 area, brain sections from rats were prepared after all recordings were finished. Images were taken from EmGFP fluorescent area to check virus transduction and then cresyl violet stained sections to confirm the position of ventral tip of traces caused by tetrodes which indicate recording sites (Figure III-5).

Figure III-5. Verification of recording sites. Two images were from the same section. The recording sites were identified with the cresyl violet staining (Left). The image on the right showed EmGFP from CA1 area injected with rAAV. Comparing two images showed the recording sites were in the virus infected area.

From those hemispheres which I managed to record unit activities from, recording sites were found to be in CA1 transduced area in 10 hemisphere of control group, 4 of Arc 92 injected hemispheres and 7 of Arc 95 injected hemispheres. In control hemispheres, fluorescence images of brain sections containing the recording sites are missing for two

rats but from the fluorescence images of neighboring sections, recording sites were surely in GFP area. In Arc 92 and Arc 95 injected hemispheres, all tetrode tips were confirmed to be in the middle of virus transduced area.

Here, I call putative pyramidal neurons recorded in the hemispheres injected with Arc92, Arc 95 and control viral vectors, as Arc 92, Arc 95 and control groups, respectively. Data from those recording sites found in the middle of virus transduced CA1 area were kept for analysis. In total, 78 putative pyramidal neurons in Control group, 51 in Arc 92 group, and 54 in Arc 95 group from the confirmed recording sites were included in the following analyses (Table III-2). The number of animals and tetrodes these neurons were recorded from are listed in the table as well.

Group	Putative pyramidal cell number	Rat number	Tetrode number
Ctrl	78	10	22
Arc 92	51	4	11
Arc 95	54	5	13

Table III-2. Neurons, animal and tetrode numbers.

III.4 Single unit properties of pyramidal neurons

III.4.1 Low firing rate in Arc knockdown pyramidal neurons through reduction of single spikes

Peak firing rate of Arc knockdown putative pyramidal neurons showed similar to controls (Figure III-6A; Arc 92, $P = 0.729$, Arc 95, $P = 0.143$, two-tailed, independent sample t test, compared with control group), but showed decreased average firing rates (Figure III-6B; Arc 92, $P = 0.160$, Arc 95, $P = 0.019$, two-tailed, independent sample t test, compared with controls). Decrease of average firing rate was significant only in Arc 95 group. Since Arc knock down efficiency is ~20% off in Arc 92 and ~50% off in Arc 95, the average rate reduction seems to reflect the extent of Arc knockdown.

In the hippocampus, pyramidal neurons exhibit action potential pattern of burst events consisting 2-6 spikes with short inter-spike intervals (≤ 6 -ms), and single spikes with > 6 -ms inter-spike intervals [171]. Burst events are thought to be generated from a large membrane depolarization due to a strong afferent synchrony, while single spikes are triggered by weak inputs from discrete events [172]. Single spike firing rate decreased in Arc knockdown groups. The reduction was correlated with Arc knockdown efficiency and only significant in Arc 95 group (Figure III-6C, Arc 92, $P = 0.251$, Arc 95, $P = 0.009$, two-tailed, independent sample t test, compared with controls). No such reduction were detected in bursts event rate (Figure III-6D, Arc 92, $P = 0.117$, Arc 95, $P = 0.500$, two-tailed, non-paired, independent sample t test, compared with control group). These

results suggest that Arc supports CA1 pyramidal neurons to fire single spikes, whereas it does not affect the generation of burst events.

Figure III-6: Decreased overall firing rate through specific reduction in single spikes.

- A.** Peak rate showed no significant differences between control and Arc knockdown groups (Arc 92, $P = 0.729$, Arc 95, $P = 0.143$, two-tailed, independent sample t test, compared with control group).
- B.** Average rate had tendency to decrease in Arc 92 group and is significantly lower in Arc 95 group than control group (Arc 92, $P = 0.160$, Arc 95, $P = 0.019$, two-tailed, independent sample t test, compared with control group).
- C.** Single spike rate decreased significantly in Arc 95 group (Arc 92, $P = 0.251$, Arc 95, $P = 0.009$, two-tailed, independent sample t test, compared with control group).
- D.** Burst event rate had no significant differences between control and Arc knockdown groups (Arc 92, $P = 0.117$, Arc 95, $P = 0.500$, two-tailed, independent sample t test, compared with control group).

* $P \leq 0.05$, ** $P \leq 0.01$

III.4.2 Pyramidal neuron spatial tuning is narrowed by the knockdown of Arc

III.4.2.1 Arc knock down pyramidal neurons are stable place cells

It is known that CA1 pyramidal neurons fire at high frequency at specific locations in a given environment, hence the name is place cells [77]. Putative pyramidal neurons recorded from control and Arc knockdown groups showed place selective firing patterns. Examples of rate maps from the three groups are shown in the Figure III-7A2. Each row represents the maps of one place cell in 6 consecutive sessions (Figure III-7A1). Place cells in all the three groups maintained a relatively stable firing pattern between sessions under the experimental conditions in which no visual sensory cue was changed. This was confirmed by quantifying the correlation of the firing pattern stability among 6 consecutive sessions. Map correlation was calculated for consecutive sessions (Figure III-7B1, 1 vs 2, 2 vs 3, 3 vs 4, 4 vs 5, 5 vs 6) and session 6 vs session 1 (Figure III-7B2). A two-way Anova was conducted to compare the map correlations (1 vs 2, 2 vs 3, 3 vs 5, 5 vs 6) among three groups (Control, Arc 92 and Arc 95). There was significant interaction of session*groups, $F(8,824) = 2.271$, $p < 0.05$. Among groups, Arc 95 has higher map correlation in session 1 compared with control and Arc 92 (B1, Arc 95 vs Ctr of session 1 vs 2, $p < 0.01$, Arc 92 vs Ctr of session 1 vs 2, $p = 0.162$; Arc 95 vs Ctr & Arc 92 vs Ctr of session 2 vs 3, 3 vs 4, 4 vs 5, $p > 0.05$; two-tailed, independent sample t test).

A1. Familiar environment

Box trial 1 Box trial 2 Box trial 3 Box trial 4 Box trial 5 Box trial 6
10 min 10 min 10 min 10 min 10 min 10 min

A2.

B1.

Figure III-7: Pyramidal neurons are stable place cells

- A. A1: Behavioral tasks. Rats underwent six 10-min sessions a day in the familiar room where they were trained prior to and after surgery. A2: Firing rate maps of putative pyramidal neurons. Each row represents one cell in six sessions. Different rows are from different groups.
- B. B1: Correlation of firing rate maps in 6 consecutive sessions (Session 1 vs 2: Arc 92, $P = 0.162$, Arc 95, $P = 0.005$; Session 2 vs 3, 3 vs 4, 4 vs 5, 5 vs 6: Arc 92, $P > 0.05$, Arc 95, $P > 0.05$, two-tailed, independent sample t test, compared with control group).
- B2: Correlation of firing rate maps compared between session 6 and session 1 (Arc 92, $P = 0.034$, Arc 95, $P = 0.004$; two-tailed, independent sample t test, compared with control group).

III.4.2.2 Arc knockdown pyramidal neurons exhibit higher spatial contrast

From recorded putative pyramidal neurons, 78 of them had place fields in the control group, 50 in the Arc 92 group and 54 in the Arc 95 group. For analysis related to place fields, only those pyramidal neurons with place fields were included. Place cell rate maps were split into place fields and non-place fields (Figure III-8A). Place field and rate were defined in method section, page 55. Quantified results showed firing rate inside place fields does not show a significant change (Figure III-8B left, Arc 92, $P = 0.980$, Arc 95, $P =$

0.161, two-tailed, independent sample t test, compared with control group). However, outside place fields, firing rate decreased significantly in the Arc 95 group, with a decreasing tendency in the Arc 92 group (Figure III-8B right, Arc 92, $P = 0.058$, Arc 95, $P = 0.004$, two-tailed, independent sample t test, compared with control group). For Arc knock down neurons, the out-to infield firing rate ratio was significantly lower in the Arc 95 group than controls, and similar trend existed in the Arc 92 group (Figure III-8D, Arc 92, $P = 0.071$, Arc 95, $P = 0.009$, two-tailed, independent sample t test, compared with control group). Thus Arc knockdown groups had larger differences in firing rates between inside and outside fields, which indicates that their firing patterns show higher spatial contrast. Visual examples of this firing pattern change are shown in Figure III-8D. While all the groups showed spatially selective firing patterns, place cells in control group tended to have a higher number of scattered spikes outside place fields. Place cells in Arc knockdown groups tended to fire only inside place fields.

Figure III-8: Higher spatial contrast in Arc knockdown pyramidal neurons.

- A. In a rate map containing the place field, average firing rates were calculated inside place field (inside the red line) and outside place field (outside the red line) respectively.
- B. Arc knockdown groups had no significant differences in firing rate inside place field (Arc 92, $P = 0.980$, Arc 95, $P = 0.160$, two-tailed, independent sample t test, compared with control group), but show lower rate in out-field regions than control group (Arc 92, $P = 0.058$, Arc 95, $P = 0.004$, two-tailed, independent sample t test, compared with control group).
- C. Lower out-to-infield firing ratio in Arc knockdown groups (Arc 92, $P = 0.071$, Arc 95, $P = 0.009$, two-tailed, independent sample t test, compared with control group).
- D. Representative firing location maps of control, Arc 92 and Arc 95 groups. All three maps contain a location selective firing pattern. In control map, more scattered spikes existed in control map, while Arc knockdown groups had spikes more concentrated in place fields.

* $P \leq 0.05$, ** $P \leq 0.01$

III.4.2.3 Reduced rate of single spikes contributes to the high spatial contrast in Arc knockdown pyramidal neurons

Among putative pyramidal neurons which had place fields, we examined the rate of single spikes and burst events in the whole map, as well as inside and outside the main place field separately. Burst event rate was similar across control and Arc knockdown groups in the whole map, within the main field and outside the main field (Figure III-9A, Arc 92, $P > 0.1$ for all three, Arc 95, $P > 0.1$ for all three, two-tailed, independent sample t test, compared with control group), but single spikes were decreased in the whole map and inside and outside the main field (Figure III-9B, Arc 92, $P > 0.05$, Arc 95, $P < 0.05$, two-tailed, independent sample t test, compared with control group), although only significant in Arc 95 group.

Figure III-9: Subfield rate decrease is contributed by reduction of single spikes.

- A. Burst event rate. Burst event rates were not significantly different among control and Arc knockdown groups when compared in the whole map, inside main field or outside main field (Whole map: Arc 92, $P = 0.117$, Arc 95, $P = 0.500$; Main field: Arc 92, $P = 0.914$, Arc 95, $P = 0.624$; Out main field: Arc 92, $P = 0.133$, Arc 95, $P = 0.102$; two-tailed, independent sample t test, compared with control group).

- B. Single spike rate. Single spike rate showed a tendency to decrease outside main field in Arc 92 group, and decreased significantly in both outside and inside main place fields in Arc 95 group (Whole map: Arc 92, $P = 0.251$, Arc 95, $P = 0.009$; Main field: Arc 92, $P = 0.980$, Arc 95, $P = 0.020$; Out main field: Arc 92, $P = 0.081$, Arc 95, $P = 0.004$; two-tailed, independent sample t test, compared with control group).

* $P \leq 0.05$, ** $P \leq 0.01$

III.4.2.4 Place field number is reduced by the knockdown of Arc

Next we calculated the spatial tuning of these place cells (putative pyramidal neurons which have place fields). A spatial tuning pattern of a place cell reflects the size and the numbers of regions a neuron is tuned to. The main place field size (the field containing the highest peak rate bin) was not different among three groups, which suggests the main spatial tuning width is not changed (Figure III-10 A1, Arc 92, $P = 0.604$, Arc 95, $P = 0.273$, two-tailed, independent sample t test, compared with control group). However, field numbers decreased in Arc 95 group (Figure III-10 A2, Arc 92, $P = 0.240$, Arc 95, $P = 0.0005$, two-tailed, independent sample t test, compared with control group), which in turn made total place field size tend to decrease in Arc knockdown place cells (Figure III-10 A3, Arc 92, $P = 0.207$, Arc 95, $P = 0.061$, two-tailed, independent sample t test, compared with control group). The differences in control and Arc knockdown pyramidal neurons indicates that the knockdown of Arc resulted in impairment of firing patterns outside of a main place field. This impairment suggests that Arc may have a role in regulating firing patterns primarily outside of a main place field (Figure III-10B), although

we cannot exclude the possibility that this effect is a secondary effect of disrupting another property of neurons which is regulated by Arc under normal conditions.

Figure III-10: Decreased spatial tuning width in Arc knockdown pyramidal neurons.

- A. A1-A3: Arc knockdown pyramidal cells had similar size for main field (Arc 92, $P=0.604$, Arc 95, $P=0.273$, two-tailed, independent sample t test, compared with control group), but less place fields (Arc 92, $P=0.240$, Arc 95, $P=0.0005$, two-tailed, independent sample t test, compared with control group) and smaller total field size (Arc 92, $P=0.278$, Arc 95, $P=0.061$, two-tailed, non-paired, independent sample t test). *** $P \leq 0.001$
- B. Map representation of Arc knock down makes individual neurons firing more spatially specific.

III.4.2.5 Spatial firing characteristics

To investigate the consequence of the decreased firing rate and altered spatial tuning pattern, spatial firing characteristics including information, selectivity, coherence and sparseness were calculated for putative pyramidal cells in control, Arc 92 and Arc 95 groups. These spatial firing characteristics of putative place cells were not significantly affected, except sparseness was significantly decreased in Arc 95 group (Figure III-11, information (Bit/Spike), information (Bit/Second), Coherence: Arc 92, $P > 0.1$ for all, Arc 95, $P > 0.05$ for all; sparseness: Arc 92, $P = 0.241$, Arc 95, $P = 0.022$; two-tailed, independent sample t test, compared with control group).

These parameters describe different aspects of a neuronal firing pattern. Information represents the ability of place cell representing the space. Coherence demonstrates the smoothness the spatial firing pattern. We did not detect an effect on these parameters. Sparseness measures how diffuse the spatial firing pattern is. Therefore, our result suggests that control group has more diffused firing pattern than Arc knockdown pyramidal neurons.

Figure III-11. Spatial firing characteristics

A-C, No significant changes for spatial firing characteristics: Information (Bit/Spike) (A), information (Bit/Sec) (B), and coherence (C), among control and Arc knockdown groups ($p > 0.05$ for all, two-tailed, independent sample t test, Arc 92 and Arc 95 groups compared with control group). D, Sparseness was significantly lower in Arc 95 group (Arc 92, $P = 0.241$, Arc 95, $P = 0.022$, two-tailed, non-paired, independent sample t test). * $P \leq 0.05$.

To avoid the variances caused by animal behaviors, a velocity threshold of 5 cm/s was applied for the analyses above. Results from the speed-controlled analysis are plotted below. No changes were observed compared with results discussed in III.4.1 and III.4.2.

Figure III-12. Single unit properties while animal run at speed >= 5cm/s.

Average rate, peak rate, field number, field size (main and sum), burst event rate, single spike rate, in field rate, out field rate and spatial contrast were calculated with a velocity of ≥ 5 cm/s.

III.4.3 Pyramidal neuron temporal tuning is narrowed by knockdown of Arc

III.4.3.1 Intact theta oscillations in virus transduced CA1 area

Theta oscillations are known to be well synchronized in left and right hemispheres. Theta waves recorded from 2 hemispheres of same animals injected with control and one of the Arc knockdown viral vectors were synchronized (Figure III-13A). No frequency differences were detected between control and Arc knockdown groups (Figure III-13B, Arc 92, $P = 0.717$, Arc 95, $P = 0.726$, two tailed, independent sample t test, compared with control group). Time of theta oscillations spent in each 30° phase bin did not have differences between control and Arc knockdown groups, indicating that theta shapes were intact in Arc knockdown region (Figure III-13D, Arc 92, $P > 0.1$, Arc 95, $P > 0.05$, two tailed, independent sample t test, compared with control group). These results show that with viral vector injection into small region in CA1, theta oscillations remain intact in the region where Arc gene was knocked down.

Figure III-13. Intact theta oscillations in Arc knockdown CA1 area.

- A. Synchrony of theta in two hemispheres. Theta oscillations from control (orange) and Arc 95 (blue) injected hemisphere revealed after filtering local field potentials with a 6-11 Hz band pass. Theta oscillations were confirmed to be synchronized between the two hemispheres.
- B. Comparison of theta frequencies from two hemispheres in same animal. No differences were detected in theta frequencies between control and Arc knockdown hemispheres (Arc 92, $P = 0.717$, Arc 95, $P = 0.726$, two tailed, independent sample t test, compared with control group).
- C. Each theta oscillation cycle was divided in 30 degree bins and time spent in each bin was quantified. The proportions of time in each 30° phase bin for theta were not significantly different between groups (Arc 92, $P > 0.1$, Arc 95, $P > 0.05$, two tailed, independent sample t test, compared with control group).

III.4.3.2 Spike tuning on theta oscillation is sharpened by Arc knockdown

In addition to spatial tuning specificity, hippocampal pyramidal neurons exhibit temporal tuning specificity to the theta oscillations. A pyramidal neuron tend to fire more spikes at its preferred theta phase than other phases [113]. To check if spikes from a pyramidal neuron have temporal tuning pattern differences, we analyzed spike distribution on theta oscillations. Under different conditions (whole map, in main field, out main field,

burst spikes, single spikes, and first spike from firing events), theta lock was calculated and significant locked neurons percentage are shown in the Table III-3. Chi-square test was applied to check whether there are differences in the percentage of cells which are significantly locked to theta oscillations. In all spike sets and first spike in event sets, Arc 92 has the significantly lower percentage of locked cells compared with control group (Figure III-14B, whole map: Arc 92 $P = 0.0003$, Arc 95 = 0.544; inside main place field: Arc 92, $P = 0.280$, Arc 95, $P = 0.102$; outside main place field: Arc 92, $P = 0.984$, Arc 95, $P = 0.569$; burst spikes: Arc 92, $P = 0.200$, Arc 95, $P = 0.649$; single spikes: Arc 92, $P = 0.613$, Arc 95, $P = 0.894$; event first spike: Arc 92, $P = 0.032$, Arc 95, $P = 0.544$; compared with control, chi square test).

Histograms of spike firing theta phases from one neuron in each group were shown in Figure III-14A. Pairwise phase consistency [166] was adopted to quantify how concentrated the spike distribution on theta phases is for the significantly locked neurons. Arc 95 locked neurons has significantly higher phase consistency compared with control, suggesting that they are more highly tuned to theta oscillations (Figure III-14C left, Arc 92 $P = 0.183$, Arc 95 = 0.029, two-tailed, independent sample t test, compared with control group).

To further understand differences in phase consistency, we quantified it separately for spikes inside place fields, outside fields, single spikes, burst spikes and first spike in firing events. For Arc 92 group, phase consistency increased only inside place fields, whereas Arc 95 group had higher consistency both outside and inside place field than control

group (Figure III-14C, inside main place field: Arc 92, $P = 0.038$, Arc 95, $P = 0.010$; outside main place field: Arc 92, $P = 0.993$, Arc 95, $P = 0.020$, two-tailed, independent sample t test, compared with control group). There was no significant difference for burst spikes theta phase consistency in all three group. For single spike, Arc 95 group almost had higher phase consistency compared with control group. To remove the bias from consecutive burst spikes, phase consistency was calculated from first spike in firing events (single spikes and first spike in bursts). Similarly, Arc 95 had significantly higher consistency (Figure III-14D, Burst spikes: Arc 92, $P = 0.592$, Arc 95, $P = 0.292$; single spikes: Arc 92, $P = 0.348$, Arc 95, $P = 0.058$; event first spike: Arc 92, $P = 0.262$, Arc 95, $P = 0.026$; two-tailed, independent sample t test, compared with control group).

Group	Whole map	In Main field	Out Main field	Burst spikes	Single spikes	Event start
Ctr	96.1	89.5	84.2	78.9	92.1	96.1
Arc92	86.3	94.1	84.3	86.3	90.2	90.2
Arc95	94.4	96.3	87.0	81.5	92.6	94.4

Table III-3. Numbers of significant theta locked neurons and spike numbers under different conditions.

A.

B.

C.

Figure III-14. Theta oscillation locking change

A. Histogram of spike theta phases in different groups. Each histogram represents spikes theta phases from one neuron in 6 consecutive sessions in one day.

B. Significantly theta locked cell percentage. Arc 92 group showed less locked cells in whole map spikes and event first spike conditions. (whole map: Arc 92 $P = 0.0003$, Arc 95 = 0.544; inside main place field: Arc 92, $P = 0.280$, Arc 95, $P = 0.102$; outside main place field: Arc 92, $P = 0.984$, Arc 95, $P = 0.569$; burst spikes: Arc 92, $P = 0.200$, Arc 95, $P = 0.649$; single spikes: Arc 92, $P = 0.613$, Arc 95, $P = 0.894$; event first spike: Arc 92, $P = 0.032$, Arc 95, $P = 0.544$; chi square test, compared with control).

C. Pairwise phase consistency of theta phase of spikes in whole map, inside main place field and outside main field from significantly locked cells (whole map: Arc 92, $P = 0.183$, Arc 95, $P = 0.029$; inside main place field: Arc 92, $P = 0.038$, Arc 95, $P = 0.010$; outside main place field: Arc 92, $P = 0.993$, Arc 95, $P = 0.020$, two-tailed, non-paired, independent sample t test).

D. Pairwise phase consistency of theta phase from of burst spikes, single spikes and first spike from firing events (Burst spikes: Arc 92, $P = 0.592$, Arc 95, $P = 0.292$; single spikes: Arc 92, $P = 0.348$, Arc 95, $P = 0.058$; event first spike: Arc 92, $P = 0.262$, Arc 95, $P = 0.026$; two-tailed, independent sample t test, compared with control group).

* $P \leq 0.05$, ** $P \leq 0.01$

III.5 Population properties of pyramidal neurons

III.5.1 Less cofiring events in Arc knockdown pyramidal neurons

Cofiring of neuronal pairs indicates the ability of the pair of neurons to interact with each other. Totally there are 109 neuronal pairs in control, 130 in Arc 92 and 101 in Arc 95 group. In both Arc knockdown groups, we found significantly lower numbers of cofiring events compared with control group (Figure III-15A, Arc 92, $P = 0.036$, Arc 95, $P = 0.034$, two-tailed, independent sample t test, compared with control group), suggesting that they were less likely to interact in the neuronal network.

To verify which spike subtype contributed to the reduction of cofiring events, we examined different categories of cofiring (burst spike vs. burst spike, single spike vs. single spike, burst spike vs. single spike). Cofiring events count did not have significant differences in burst vs burst (Figure III-15B, Arc 92, $P = 0.368$, Arc 95, $P = 0.377$, two-tailed, independent sample t test, compared with control group). In single spikes vs. single spikes, cofiring tended to decrease in Arc 92 group and was significantly decreased in Arc 95 group compared with control group (Figure III-15C, Arc 92, $P = 0.065$, Arc 95, $P = 0.008$, two-tailed, independent sample t test, compared with control group). Cofiring events between burst spikes and single spikes decreased significantly in Arc 92 group, not in Arc 95 group, compared with control (Figure III-15D, Arc 92, $P = 0.018$, Arc 95, $P = 0.239$, two-tailed, independent sample t test, compared with control group).

Normalized count of each categories of cofiring counts showed that single spikes vs. single spikes contributed most to the total cofiring events (Figure III-15E).

Figure III-15. Cofiring events count.

- A. Cofiring counts of all spikes. Arc knockdown groups had significantly less cofiring events than control group (Arc 92, $P = 0.036$, Arc 95, $P = 0.034$, two-tailed, independent sample t test, compared with control group).
- B-D. Cofiring counts decreased in Arc knockdown groups in spike subtypes containing single spikes. Burst spikes v.s. burst spikes (Figure III-15B, Arc 92, $P = 0.368$, Arc 95, $P = 0.377$, two-tailed, independent sample t test, compared with control group). Single spikes v.s. single spikes (Figure III-15C, Arc 92, $P = 0.065$, Arc 95, $P = 0.008$, two-tailed, independent sample t test, compared with control group). Burst spikes v.s. single spikes (Figure III-15D, Arc 92, $P = 0.018$, Arc 95, $P = 0.239$, two-tailed, independent sample t test, compared with control group).
- E. Percentage of each categories of cofiring events. Note single spike vs. single spike cofiring contributed the most while burst spike vs. burst spike took the least proportion in all groups.
- * $P \leq 0.05$, ** $P \leq 0.01$

III.5.2 Worse decoding ability of Arc knockdown pyramidal neurons

To check the ability of the population to represent space, we examined the distance of estimated location conveyed from multiple neurons, to the rat's real location (Figure III-16A, black dot: real position, red dot: estimate position). Arc knockdown putative pyramidal ensembles had much larger decoding errors compared with control group (Figure III-16B, Arc 92, $P=0$, Arc 95, $P=0$, two-tailed, independent sample t test, compared with control group).

To remove the bias from different neuron numbers in each group, the same number of neurons were randomly chosen to calculate decoding error. Likewise, Arc knockdown ensembles had significantly higher errors in estimating locations (Figure III-16C, Arc 92, $P=0.004$, Arc 95, $P=6.6 \times 10^{-64}$, two-tailed, independent sample t test, compared with control group).

Figure III-16. Position decoding.

- A. Examples of position decoding in different groups. Black dots are real positions while red dots are estimated positions by decoded by recorded data.
- B. Decoding errors. Arc knockdown pyramidal neuron ensemble had much higher decoding error distances to real positions than control ensemble (Arc 92, $P=0$, Arc 95, $P=0$, two-tailed, independent sample t test, compared with control group).
- C. Decoding errors with same size ensemble. With the same number of neurons in the ensemble to decode the position, Arc knockdown groups remained significantly higher errors than control group (Arc 92, $P=0.004$, Arc 95, $P=6.6 \times 10^{-64}$, two-tailed, independent sample t test, compared with control group).

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

IV Discussion

IV.1 Arc regulates neuronal activity by increasing sensitivity to small input

A decrease in Arc expression leads to a decrease in place cell firing rate

We found that Arc knockdown place cells have lower firing rate compared with control (Figure III-6), which suggests that Arc has a role in increasing the firing rate of place cells.

Changes in firing rate can be due to changes in 1) input activity from presynaptic neurons, 2) synaptic strength, 3) the ability of input summation, 4) intrinsic cellular excitability (i.e. threshold potential) or a combination of these possibilities.

Since our virus transduction and Arc knockdown was limited in the CA1 area, changes in input activity is unlikely to be the cause. Considering the known role of Arc in regulating synaptic functions, synaptic strength and the ability of input summation would be the more likely cause for the observed reduction in firing rate of place cell activity.

We found that burst firing generation (Figure III-6) is similar in all groups. This finding indicates that it is unlikely that intrinsic excitability was affected by Arc knockdown pyramidal neurons. This is supported by an in vitro study [173]. In this study, transgenic mice with Arc promoter driven GFP was used to differentiate Arc expressing and non-expressing neurons in the brain. After Arc induction, Arc positive and negative neurons can be distinguished by checking GFP signals on brain slices. They found that Arc-positive and -negative CA1 pyramidal neurons have similar passive membrane properties and action potential threshold. With the same amount of depolarizing currents injected, the

firing rate between Arc positive and negative CA1 pyramidal neurons are comparable as well. These findings suggests that Arc does not regulate neuronal intrinsic excitability.

A decrease in Arc reduces single spike generation but not burst firing

We found that in Arc knockdown pyramidal neurons, only the firing rate of isolated, single spikes was significantly reduced while burst events rate remains unchanged (Figure III-6). Thus the overall firing rate reduction caused by Arc knockdown reflected specific reduction in single spikes. To generate burst spikes, large membrane depolarization associated with dendritic spike is required [174]. Similar depolarization in soma area with dendritic depolarization lower than threshold only induced single spike [174]. Dendritic spikes arise as a result of regenerative Na^+ and/or Ca^{2+} channel activation that begins in the dendrites. Its initiation threshold is higher than that generated in axons, suggesting they require stronger input [175]. A moderate input summed from synapses without generating dendritic spike elicits single spikes more likely than burst event. Arc is known to regulate spines morphology [66] and synapse efficacy [30]. Its role in dendritic function has not been reported. The regulation of synapses by Arc might change the summation of inputs without affecting the mechanism underlying the generation of dendritic spikes, and increase single spike rate. In vivo study of burst generation in hippocampal pyramidal neurons suggest that in addition to the sufficient level of excitation, a preceding silence is required [176]. This study found the probability of burst initiation is largest with a 100 ms – 1 s silence. With the increase of

single spikes occurrence in control group in our experiment, no differences in burst generation was detected. This suggests that the single spike manipulation by Arc does not fall into the critical temporal time window of 100ms – 1s ahead of bursts.

For a single neuron, bursts and single spikes are thought to encode different stimulus features [177]. Taken stimulus frequency as an example, burst firing happens during more specific stimulus frequency, while single spikes occur at the entire range of stimulation [178] [177]. This is in line with the idea that bursts encode narrower input range but single spikes cover larger range. The increased single spikes in Arc activated neurons suggest an increased ability in coding wider range of inputs.

Loss of Arc decreases in- to-out field firing rate ratio and narrows neuronal tuning

We found that most hippocampal pyramidal cells showed location specific firing patterns in control and Arc knockdown (Figure III-7) groups. Among these neurons, spikes outside place field were significantly reduced by Arc knockdown, while in-field firing rates remained intact (Figure III-8). This result indicates that Arc has a biased role for regulating the firing of place cells outside their place fields. Spikes outside a place field have been proposed not to have any particular function but noise. However, a study has started to unveil a role for these out-field spikes. The study found that by aversive learning on a maze, a population of place cells shifted place fields toward where many out-field spikes happen [179], indicating that out-side field spikes have functional

significance determining potential future spatial positions. Our study shows that these out-field spikes are decreased in the absence of Arc. This offers a molecular explanation for generating these out-field spikes. Further, the existence of regulation suggests they have some function, but not likely to be a noise.

We also found that while the main place field with the highest peak firing rate was intact in size and firing rate, the total number of place fields was reduced by Arc knockdown (Figure III-10). This reduction means that non-main place fields with a smaller peak were lost with Arc knockdown. Together with selective reduction in firing rate outside place fields, this result indicates that Arc knockdown compromised the spatial tuning of place cells. Thus our results suggest that Arc has a role in broadening spatial tuning of place cells.

There are genetic and pharmacology studies manipulating genes/molecules such as NMDA receptor, AMPA receptor, CaMKII α and tested their effects on CA1 place cell activity. CA1 place cells were found to have more diffused spatial distribution in NMDA receptor knock out mice. In an AMPAR knockdown study, place cells were found to have slower remapping speed and larger field size compared with control. In another study, the blockade of CaMKII α leads to less precise and less stable place cells.

The published results all showed that blocking the natural activation or synthesis of a certain molecule abolishes place cell activities in some way (expand place fields, low spatial specificity, delay field remapping etc). Our study showed results in an opposite

way that blocking the natural Arc activation causes a more spatially specific firing pattern. Also, our study is the first that found a molecule selectively controls spikes outside place field in a place cell.

Loss of Arc narrows the spike distribution on theta oscillation

Place cells have temporal firing patterns related to theta oscillations. Spikes from a place cell are locked to certain phase, which means spikes show more densely distribution around certain theta phase [113]. Our results showed that CA1-specific Arc knockdown does not change theta oscillation frequency and phase duration. On the other hand, we found that Arc knockdown made place cells have narrower spike distribution on theta oscillations (Figure III-14), which suggests that Arc reduces the extent of phase lock to theta oscillations or in other words broaden a temporal tuning of place cell firing on theta oscillations.

In addition to theta phase locking, a related phenomenon called theta precession is observed in place cells. When animal travels through the place field of a given place cell, the first spikes of the place cell tend to fire at the late phase of the ongoing theta oscillations, and following spikes tend to fire at earlier phases compared with previous spikes. When the place cell exits its place field, spikes are near the beginning of theta cycles [113] [114]. The gradual phase differences of place cell spikes on theta oscillations based on the travelling distances inside place field could offer additional spatial

information of where the animal is inside a place field. With Arc activation, the distribution of spikes on theta oscillations are wider, which might increase the ability for temporal characteristics of place cell firing to represent the additional spatial information.

Arc potentially increases weak input induced spikes

As I discussed in the previous sections, Arc knockdown reduced single spikes, out-field spikes and non-preferred theta phase spikes. This biased effects on subpopulations of spikes but not others suggests that the regulation of Arc over place cell firing is not a cell-wide mechanism such as intrinsic excitability, but a more specific mechanism such as the strength or summation of specific synapses. Single spikes, out-field spikes and non-preferred theta phase spikes occur under the conditions associate with lower firing rate and are generally considered to be produced when neurons receive weak inputs [103] [180]. Therefore, our results overall can be interpreted as that Arc may facilitate place cell firing in response to weak input. There are two possible explanations of the facilitated neuronal response to weak input from a synaptic view. First is that individual synapses receiving weak input are enhanced with stronger transmission efficacy. However, this possibility is unlikely to be correct because it has been shown that Arc expression increases AMPAR endocytosis [30] and reduce synapse strength [32]. Another possibility is that the summation of synapses receiving weak input is enhanced by Arc mediated synaptic regulation. For example, Arc was found to inversely tag inactive

synapses and remove or inhibit these unwanted synapses [53]. By such regulation, existing synapses could have a better efficiency in summing weak inputs and generating action potentials.

IV.2 Arc activation increases population function

Arc knockdown decreases probability of neuronal interactions

We found that Arc knockdown reduced the frequency of co-firing between place cells (Figure III-15), implicating that Arc facilitates co-activation of place cells. For a group of neurons to function together, they need to be co-activated [181]. Cofiring of neurons increases the information stability. For information encoded by a single neuron varies with the fluctuation of neuronal firing rate. This fluctuation is counteracted while multiple neurons are involved. Thus information coded by cofiring of neurons are more stable [182] [183] [184]. Our results showed Arc knockdown place cells has less single spike involving cofire events. This reduction is associated with the rate decrease in single spikes. This results suggests that Arc expression improves the functional interaction among neuronal population primarily by facilitating single spike generation.

Loss of Arc decreases population spatial representation ability

The activity of individual neurons has limited information. For a single place cell, by showing the firing rate of a known place cell, it is difficult to tell the accurate location of the animal. Locations reconstructed from the firing patterns of a group of place cells have higher accuracy [185]. We found that Arc knockdown reduces the accuracy of position decoding from the activity of neuronal population (Figure III-16). This finding suggests population spatial representation ability is better with Arc expression. We found that more spikes occur outside place field in Arc expressing place cells, which suggests that Arc expression may reduce spatial specificity of firing of individual neurons. However, we observed that these neurons have better accuracy in population spatial representation.

In neural network model studies, there were controversy on whether a stable and finely tuned single neuronal firing pattern, which mean high specificity to input, is necessary for a neural network to function efficiently [186] [183] [187]. Our experimental data showed a finely tuned single neuronal activity is not required for an efficient network information representation. On the contrary, extremely narrow or fine single neuronal tuning might be harmful for population reliabilities. Therefore, Arc-mediated reduction in tuning specificity may improve network functions by facilitating interaction among neuronal population.

IV.3 Arc bridges experience with neuronal activity and potentially contribute to memory engram

Neurons are highly adaptable, encoding new information when needed and retaining learned information in resistance to the modification of molecules and synapses. Our experiment showed Arc knockdown reduced firing rate and firing specificity. This reduction revealed that Arc acts as bridge between experience and modification of neuronal firing patterns.

How does Arc-regulated neuronal activity contribute to learning and memory? Arc knockout mice have normal learning ability and abolished long-term memory [58]. According to the study of memory engram cells, neuronal population activated during information encoding failed to be reactivated by long-term memory retrieval [188] [18]. Our results suggests, with Arc expression, neurons have higher neuronal activity with broader tuning and improved network function.

Arc expression happen in memory engram cells [73]. Does the higher, broader neuron activity from Arc activation contribute to the neurons to be assigned into a memory engram? Studies over expressed cyclic AMP-responsive element binding protein (CREB) in 15- 25% of lateral amygdala neurons. After memory-related task was introduced to the animal, memory recall happened specifically when reactivating those neurons with elevated level of CREB, compared with other neuron group [189] [190]. CREB is a transcription factor whose phosphorylation is triggered by various signaling pathways

following synaptic activity [191] [192]. Phosphorylated CREB upregulates target gene transcription including Arc [193] [194]. Our results suggest that Arc expression leads to high, broad-tuning neuronal activity. This change mediated by Arc expression might be downstream to CREB-mediated regulation, and therefore may also regulate engram cell assignment.

To further understand how Arc contributes to the memory, results from c-Fos positive engram cells gave us hints. A study in 2018 found there are c-Fos positive and c-Fos negative place cells [195]. c-Fos positive place cells have higher average firing rates but similar peak rates, and lower spatial specificity, which interestingly have similarity to the properties supported by Arc expression, suggested by our results. Is this similarity because c-Fos positive cells acquire the firing characteristics through an Arc-mediated mechanism? Or is it because memory encoding requires the common firing characteristics which was observed in c-Fos expressing cells and was suggested by our study, although these two are mechanistically independent? This issue needs to be clarified in future study.

IV.4 Future work

Our work bridges the activity-dependent gene expression with neuronal activity change. With the findings that Arc increases AMPAR endocytosis [30] and reduce synapse strength [196] [53], it is not intuitive that neuronal activity is increased and broadened by Arc-mediated regulation. However, the relationship between synaptic activation and neuronal firing is known to be complex. Membrane potential changes caused by multiple synapses at different timing summates and are affected by membrane properties, resulting in a particular pattern of neuronal firing. Regarding such complex interaction among synapses, Arc selectively weakens weakly-activated, unwanted synapses in the network to increase the efficiency of remaining synapses [53]. Or the removal of inactive synapses may make a room for forming new neighboring synapses which can cooperate with existing synapses to fire the neuron. Whatever the mechanism is, our work offered a new insight of synaptic regulation and neuronal activity, indicating that syntactical level enhancement or decline does not necessary cause same change in neuronal activity. More future work examining the functional relationship among synapse regulating signaling pathway, synaptic function and behaviorally-relevant neuronal activity, is required to understand the how synaptic regulation contribute to neuronal activity.

Deficits in memory was found in Arc knockout mice [58]. The cellular mechanism is not understood yet. Our results suggested that Arc activation increases and broadens single neuronal activity, and enhances network functions. However, how these changes,

probably occurring in engram cells, results in memory formation or mediate memory storage and retrieval is still far from full understanding. Further research on this topic requires characterization of how neuronal firing codes memory-related information, how changes in such activity in engram cells corresponds to memory formation, storage and retrieval.

Together with other immediate early genes [20] [195], Arc is used as a marker for memory engram cells. We showed that Arc increases, broadens single neuronal activity and enhances network function, which might support neuronal function in a memory. There are many other immediate early genes and many of them are likely to be involved in memory formation and maintenance. How they contribute to the memory remain to be studied. It is important to understand whether, and if so how, each of such genes/proteins is involved to fully understand the molecular mechanism of memory formation and storage.

The new discovery that Arc transporting its genetic information to other cells offers a new insight of cellular communications [75] [74]. It remains unknown that whether Arc protein only transport Arc mRNA or likewise mRNA encoding different proteins. Together with role of Arc in regulating neuronal activity and synaptic plasticity, this transportation might have an impact on network functions.

V References

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