

Behaviour-dependent activity and synaptic organisation of septo-hippocampal GABAergic neurons

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Abstract

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Animal behaviour is coordinated by biological rhythms over multiple timescales. In the temporal cortex, rhythmic oscillatory activity in the 5 to 12 Hz theta frequency range supports behaviour through temporal windows of changes in excitability and circuit computation. Subcortical inputs, including those from the medial septum are thought to generate the theta oscillatory activity. However, the rules of innervation of identified types of cortical cells in different cortical regions by a diverse population of septal neurons are largely unknown. During my DPhil studies, I tested the hypothesis that medial septal GABAergic neurons which exhibit distinct activity patterns would express distinct molecular marker combinations and have distinct synaptic partners.

In *Chapters 1* and *2*, I formulate my research questions and explain the methodology used. My main findings are presented in chapters 3 to 7. In *Chapter 3*, I present the strategy used to identify four populations of rhythmic medial septal neurons based on their spike-train dynamics in the mouse *in vivo*. In *Chapter 4*, I present a detailed analysis of the spike-timing and molecular parameters of a specialised population of rhythmic neuron, the *Teevra cells*. Teevra cells are PV⁺, do not change their firing rate from rest to running and fire short bursts of action potentials preferentially at the trough of both CA1 theta and slow irregular activity recorded in stratum pyramidale. In *Chapter 5*, I show that Teevra cells selectively innervate axo-axonic cells and CCK-immunopositive interneurons in hippocampal area CA3, bypassing CA1, CA2, and the dentate gyrus. In *Chapter 6*, I show that Teevra cell axons terminate in restricted septo-temporal segments of CA3 according to their rhythmicity. Finally, in *Chapter 7*, I present the *Komal cells*, which fire long bursts of action potentials at the peak of theta, increase their firing rate during running, and preferentially innervate extrahippocampal cortices. I discuss the broad relevance of my observations in *Chapter 8*.

Overall, my results show an unexpected specialisation in the organisation of medial septal GABAergic inputs to the temporal cortex. *Teevra* cells fire maximally at the trough and preferentially innervate the CA3 whereas *Komal* cells fire maximally at the peak and target extrahippocampal cortices, thereby coordinating the timing of converging glutamatergic inputs to CA1 via disinhibition. This cortical-region-specific GABAergic innervation by physiologically distinct septal neuronal types suggests a general mechanism for the coordination of network activity, both during regular theta oscillations and irregular large amplitude waves, providing windows of computation through synchronisation of neuronal activity in the cortex.

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List of abbreviations

A

AAC, axo-axonic cells

AIS, axon initial segment

C

CA1, Cornu Ammonis 1

CA2, Cornu Ammonis 2

CA3, Cornu Ammonis 3

CB, calbindin

CCK, cholecystokinin

CR, celretinin

Cy3, cyanine 3

Cy5, cyanine 5

D

DAB, 3,3'-diaminobenzidine

DG, dentate gyrus

E

EC, entorhinal cortex

EEG, electroencephalography

EM, electron microscopy

G

GABA, gamma-Aminobutyric acid

GABAA, gamma-Aminobutyric acid type

A receptor

GABAB, gamma-Aminobutyric acid type

B receptor

H

HCN, hyperpolarization-activated cyclic nucleotide-gated channel

HRP, horseradish peroxidase enzyme

I

ISI, inter-spike intervals

IQR, inter quartile range

L

LED, light-emitting diode

LFP, local field potential

LIA, large amplitude irregular activity

LTD, long term depression

LTP, long term potentiation

M

mGluR1a, metabotropic glutamate receptor 1a

MS, medial septum

N

NHS, normal horse serum

NMDA, N-methyl-D-aspartate receptor

NOS, nitric oxide synthase

NPY, neuropeptide tyrosine

O

O-LM, oriens-lacunosum moleculare

OF, oscillatory frequency

P

PB, phosphate buffer

PV, parvalbumin

PrS, presubiculum

PaS, parasubiculum

R

RI, rhythmicity index

S

s.d., standard deviation

SATB1, special AT-rich sequence-binding protein 1

SST, somatostatin

SWR, sharp wave associated ripple

T

TBS-TX, tris buffered saline with Triton

X-100®

V

VIP, vasoactive intestinal polypeptide

VGAT, vesicular GABA transporter

VGluT2, vesicular glutamate transporter

Chapter 1: General Introduction

Coordination of neuronal activity across brain regions is essential for behaviours such as learning, memorising, and goal-directed movement (McNaughton et al., 2006; Singer, 1993; Staudigl and Hanslmayr, 2013; Winter et al., 2013). Precision in coordination is mediated by distinct cell types across distinct brain regions, which likely evolved to represent the inner and outer world in terms of physical mechanisms of the brain. Understanding how diverse cell types perform specialised roles during well-defined behaviours is necessary to explain not only how the normal brain functions but also what goes wrong during neurological and psychiatric diseases. In my DPhil, I have explored the physiological and anatomical organisation of long-range GABAergic connections to study how they support temporal coordination across brain regions.

1.1 Neuronal correlates of spatial memory

The dorsal hippocampus is involved in the formation, storage, and recall of episodic memories through experience dependent formation of neuronal assemblies. During active exploration of an environment, ‘place cells’ in the hippocampus are thought to represent different locations forming a spatial map by discharging bursts of action potentials in temporal sequences maximally at their ‘place field’ (O’Keefe and Dostrovsky, 1971; O’Keefe and Recce, 1993). Since the discovery of place cells, a variety of neurons with spatially modulated activity have been discovered in the temporal lobe, which is perhaps the most intensively studied cortical region. These include the ‘head direction cells’ concentrated in the presubiculum (PrS), which fire maximally corresponding to directions the animal faces irrespective of its location (Taube et al., 1990), ‘grid cells’ and ‘border cells’ in the entorhinal cortex (EC), which tile the space into periodic triangular vertices and represent environmental boundaries, respectively (Fyhn et al., 2004; Solstad et al., 2008). Recent studies also show an internal representation of time by

‘time cells’ (Eichenbaum, 2014; Pastalkova et al., 2008). These representations in the PrS, parasubiculum (PaS), EC and hippocampal regions are facilitated by interconnected glutamatergic cortico-cortical projections (Swanson and Cowan, 1977) and relatively sparse long-range GABAergic connections (Melzer et al., 2012). Based on the above, it is widely suggested that the hippocampal formation is essential for spatial navigation when new spatial locations are being encoded for memory guided behaviours. But these distinct representations are not enough to explain behaviour, which in addition to these representations would also require a temporally coordinated activity of spatially modulated cells in dispersed cortical regions. For instance, to correctly take a left turn at an intersection or a T-junction, the above representations of space need to be temporally coordinated according to relevant behavioural timescales; the intersection being represented as a ‘place’, ‘boundary’, ‘grid’, and ‘head-direction’. In this thesis, I explore the cellular and synaptic mechanisms responsible for coordinating ‘brain-time’ across multiple brain regions.

1.2 Temporal organisation of hippocampal activity

The hippocampus has been suggested to associate life events on several timescales in temporally determined sequences of neuronal assemblies. Such temporal coordination includes theta oscillations (~5 – 12 Hz), which are most prominent during movement and REM sleep (Kramis et al., 1975; Winson, 1972), gamma oscillations (~30 – 120 Hz), which are phase-amplitude coupled to theta (Colgin et al., 2009; Bieri et al., 2014; Lasztóczy and Klausberger, 2016; Schomburg et al., 2014) and hippocampal sharp-wave ripple oscillations (SWRs, 130 – 230 Hz) mainly occurring during slow wave sleep, awake immobility and consummatory behaviour (Buzsáki, 1986; O'Keefe and Nadel, 1978). During immobility, large amplitude irregular activity (LIA) dominates the hippocampal field potential (O'Keefe, 1978). During voluntary locomotion, theta oscillations are the most prominent electrophysiological signature in the hippocampus (Buzsáki, 2002; Vanderwolf, 1969). Current source density analysis reveals

that theta oscillations are generated mainly by the entorhinal cortical input (perforant path), CA3 Schaffer collateral and commissural pathways, and voltage-dependent Ca^{2+} current in pyramidal cell dendrites (Brankack et al., 1993; Kamondi et al., 1998; Magee and Johnston, 1995; Buzsaki, 2002; Buzsaki et al., 1986; Lasztóczy and Klausberger, 2014). This oscillatory activity represents temporal windows for local circuit computation in the CA1 and CA3 (Buzsaki and Draguhn, 2004; Mizuseki et al., 2009), and thus is critical for encoding and decoding by modification of synaptic efficacy. An influential model of pyramidal cell synchronisation in the hippocampal CA1 area suggests that *encoding* of new sensory information is driven around the peak of the theta cycle (as referenced to the pyramidal cell layer), corresponding to the entorhinal cortical input when plasticity is strongest, and *retrieval* of stored contextual associations is around the theta trough, which corresponds to the maximal CA3 input (Hasselmo et al., 2002). This theory has received support from electrophysiological, behavioural, and pharmacological experiments (Bieri et al., 2014; Douchamps et al., 2013; Wells et al., 2013). The glutamatergic inputs arriving to the hippocampal area CA1 follow an oscillatory organisation. The input from the entorhinal cortex layer 3 (EC3) is maximal at the peak and from CA3 is maximal at the trough relative to dorsal CA1 theta oscillations in stratum pyramidale (Mizuseki et al., 2009). Both inputs make synapses directly on pyramidal cells as well as on a highly diverse family of GABAergic interneurons. *What are the mechanisms that underlie coordinated and temporally precise organisation of inputs in the hippocampus?*

1.3 GABAergic neurons coordinate temporal activity in the hippocampus

The behaviourally-dependent activity of a relatively uniform group of pyramidal cells in the hippocampus is supported by a rich diversity of GABAergic interneurons, which constitute at least 21 distinct cell types based on anatomical, physiological, and molecular analyses in CA1 and CA3 (Cobb et al., 1995; Klausberger et al., 2003; Klausberger and Somogyi, 2008; Somogyi, 2010; Ylinen et al., 1995). These GABAergic neurons show remarkable differences

in their dendritic and axonal arborisations reflecting their differential connectivity, which determines their influence on the network. For instance, axo-axonic cells (AACs) preferentially innervate the axon-initial segment of pyramidal cells (Somogyi et al., 1983; Viney et al., 2013) and parvalbumin (PV)-immunopositive basket cells terminate on pyramidal cell somata and proximal dendrites in a basket-like manner. Additionally, these two PV⁺ cell types of the hippocampus could be differentiated using a transcription factor, SATB1 (Viney et al., 2013). During movement, AACs and PV⁺ basket cells fire with similarly high frequencies but on complementary temporal segments of the descending slope of theta cycles (Klausberger et al., 2003; Lapray et al., 2012; Tukker et al., 2013a; Viney et al., 2013). Similarly, other GABAergic cell types have distinct connectional and temporal activity architecture (Katona et al., 2012; Katona et al., 2017; Lapray et al., 2012; Tukker et al., 2013b; Varga et al., 2012; Varga et al., 2014). Together, these neurons contribute to a temporal redistribution of inhibition across all compartments of pyramidal cells, from the AAC mediated inhibition at the axon initial segment, to the basket cell innervation at the soma, and others all the way to the distal dendrites of principal cells during theta oscillatory cycles (Somogyi et al., 2014). Firing phase preference is likely to be an important factor in determining the influence of a particular neuronal population since it correlates with the distinct glutamatergic inputs arriving to the hippocampal area CA1. This may explain that optogenetically activating PV⁺ population at distinct phases of theta can alter encoding and retrieval functions (Siegle and Wilson, 2014). *Which are the long-range neurons and pathways that support the temporal differentiation of the cortical GABAergic neurons?*

1.4 Role of the medial septum in theta coordination

The medial septum sends axonal projections to the hippocampus via GABAergic (Freund and Antal, 1988a; Gaykema et al., 1990; Kohler et al., 1984), glutamatergic (Fuhrmann et al., 2015; Justus et al., 2017; Sotty et al., 2003), and cholinergic projections ((Lewis and Shute, 1967);

reviewed by (Gielow and Zaborszky, 2017)). Inactivation of the medial septal nucleus results in a loss of theta oscillatory activity in the hippocampus (Winson, 1978) and many studies have examined behavioural performance and activity of cortical neurons and LFPs in the absence of medial septal input (Brandon et al., 2011; Mizumori et al., 1990; Mizumori et al., 1992; Mizumori et al., 1989). The GABAergic projections from the medial septum preferentially innervate GABAergic neurons in the hippocampus (Freund and Antal, 1988). Recently, this remarkable selectivity was also confirmed in extrahippocampal cortices (Gonzalez-Sulser et al., 2014; Unal et al., 2015). This raises the possibility that the activity patterns of medial septal neurons in awake behaving animals would correlate with the differences in the activity patterns of their target GABAergic neurons. *In vitro*, septal neurons have been shown to modulate the firing of hippocampal pyramidal cells and interneurons (Huh et al., 2010; Leão et al., 2012; Tóth et al., 1997). *In-vivo*, some medial septal neurons exhibit bursts of action potentials tightly phase coupled to ongoing theta oscillations (Gogolak et al., 1968; Petsche et al., 1962; Ford et al., 1989), and hence they are often referred to as ‘pacemaker’. However, in addition to strongly rhythmic neurons, diverse rhythmic and non-rhythmic activity patterns of medial septal neurons were observed under anaesthesia (Borhegyi et al., 2004; Simon et al., 2006) and in un-anaesthetised rats (Dragoi et al., 1999; King et al., 1998). In other areas of the basal forebrain, cholinergic neurons and glutamatergic neurons are thought to influence cortical activation, such as mediating the network and behavioural state transition from sleep to wakefulness (Cape et al., 2000; Huerta and Lisman, 1993; Xu et al., 2015). These seem to be arrhythmic firing in *substantia innomata*, and their activity is distinctly correlated to sleep-wake cycle (Hassani et al., 2009). Lesions or inactivation of the MS cholinergic neurons reduce theta power but does not abolish it (Lee et al., 1994; Carpenter et al., 2017) and muscarinic receptor blockade in the MS abolishes the correlation of theta frequency with running speed (Newman et al., 2013). Additionally, during a behavioural task which required sustained attention, the cholinergic basal

forebrain neuronal responses were fast (approximately 18 ms) suggesting that they broadcast a rapid and precisely timed reinforcement signal across cortical areas (Hangya et al., 2015). Optogenetic activation of glutamatergic neurons at theta frequency can also pace hippocampal theta oscillations (Fuhrmann et al., 2015), however the effect is likely mediated through local MS connections (Robinson et al., 2016). The activity patterns of identified medial septal cholinergic and glutamatergic neurons are not known.

A large proportion of rhythmic neurons were shown to express the calcium binding protein parvalbumin (Borhegyi et al., 2004; Simon et al., 2006), however not all of them ‘lead’ or ‘pace’ the changes observed in the hippocampal LFP (Hangya et al., 2009) and some might be ‘followers’ receiving rhythmic input from hippocampo-septal projection neurons (Toth et al., 1993). Further, since the theta bursting neurons were shown to have extensive local axonal arborisations in the septum, it was suggested that synchrony between the septal GABAergic ‘pacemaker’ units may be brought about by their recurrent collateral interactions (Borhegyi et al., 2004). The ‘pacemaker’ concept is challenged by the observation that the percentage of rhythmic bursting neurons is higher under anaesthesia than without it (Lee et al., 1991). This suggests that septal neurons do not ‘generate’ or ‘pace’ theta rhythmic burst firing in the awake animal on their own and that their activity patterns are affected by the ‘awake’ state, likely integrating the rhythmic inputs from the subcortical theta rhythm generating network involving the brainstem, thalamus, and hypothalamus (Vertes and Kocsis, 1997). The diagram I constructed taking into account some of the connections (see Fig. 1.1 for a list of septal inputs and outputs) suggests that instead of a ‘pacemaker nucleus’ the medial septum is a ‘relay junction’. *What are the activity patterns of medial septal neurons in awake behaving animals and how do they coordinate theta oscillatory activity?*

There are medial septal GABAergic, glutamatergic, and cholinergic projections to all of temporal lobe cortical areas, but the synaptic target regions and postsynaptic cells of single

septal neurons are not known, except for cholinergic neurons (Wu et al., 2014), which have widespread collaterals to many areas in the cortex. A striking yet underappreciated feature of GABAergic septal afferents to the hippocampus is the extensive targeting of interneurons in CA3, the hilus, and granule layer of the dentate gyrus (DG) compared to CA1 (Freund and Antal, 1988). This is of particular relevance as the bilateral projections of CA3 pyramidal cells form an auto-associative network (Bennett et al., 1994; Witter, 2007). Additionally, the CA3 and DG are thought to be involved in distinct aspects of spatial coding (Neunuebel and Knierim, 2014), raising the possibility that they might receive distinct septal inputs. *Do septo-cortical long-range projecting neurons follow target-region-specific axonal distributions and cell-type-specific theta-phase firing preferences?*

The lack of knowledge on how different types of rhythmic bursting septal neurons contribute to cortical circuits is due to the paucity of information on the relationship between the topography of their cortical projections and activity patterns in awake animals.

1.5 Behavioural effects of medial septum inactivation

Since the discovery of place cells (O'Keefe and Dostrovsky, 1971) and the identification of the relationship of their firing to theta oscillations (O'Keefe and Recce, 1993) coupled with the theta rhythmic burst firing of medial septal neurons (Petsche et al., 1962; Gaztelu & Buno 1982; Alonso et al., 1987., King et al., 1998; Ford et al., 1989), a variety of manipulations of the medial septal nucleus have been performed to assess spatial memory deficits. Inactivation of the medial septal nucleus by electrolytic lesions results in a loss of theta oscillatory activity in the hippocampus (Winson, 1978) and is also accompanied by spatial memory deficit. Intraseptal administration of GABAergic agonist, muscimol produces memory impairments in radial-arm-maze task (Chrobak et al., 1989). This muscimol-induced impairment is also observed with the introduction of a 4 hr delay, suggesting that the behavioural deficit reflects an inability to store or retain spatial working memory rather than a general performance deficit (Chrobak et al.,

1989). Similar spatial working memory impairment was observed with muscimol injections before the rats entered the Morris water maze (Brioni et al., 1990). The behavioural effects of medial septal inactivation were not only limited to spatial working memory. Categorically testing medial septal contribution to working and reference memory in continuous-conditional-discrimination and sensory-discrimination tasks showed that muscimol and scopolamine infusions in the MS impaired working memory but not reference memory (Givens and Olton, 1994). In the same task, electrophysiological changes observed upon microinfusions correlated with errors in working memory suggesting that the septo-hippocampal pathway is critical in the timing of working memory processes (Givens and Olton, 1994). Interestingly, medial septal activation by infusions of cholinergic agonist carbachol also impairs the retention of memory in a delay-non-match-to-sample-radial-arm-maze task (Bunce et al., 2004), suggesting that merely the activation of a particular subpopulation is not enough for encoding, retrieval or long term retention of memory.

Overall, these studies indicate that the medial septal nucleus is critical for working memory and further warrant the examination of the activity of distinct subpopulations of medial septal neurons in awake behaving drug-free animals to understand their contribution to temporal coordination in cortical areas.

1.6 Aims

During my DPhil studies, I attempted to dissect the contribution of distinct medial septal GABAergic cell types to different network and behavioural states at single cell resolution. I tested the hypothesis that medial septal GABAergic neurons which exhibit distinct activity patterns would express distinct molecular marker combinations and terminate on different synaptic targets. I have used a combination of extracellular multi-unit recording, targeted single neuron recording and juxtacellular labelling (Pinault, 1996) in behaving head-fixed mice to reveal the rules of septo-hippocampal connectivity.

My results contribute to an organisational framework of medial septal GABAergic inputs to the temporal lobe. Briefly, I have:

- performed extracellular recordings using tetrodes and glass electrodes to determine the diversity of medial septal neuronal activity patterns in awake behaving mice.
- correlated the activity patterns observed in the septum to the local field potentials (LFPs) in the hippocampus.
- identified distinct subpopulations of medial septal rhythmic neurons using parameters based on the spike-train dynamics.
- targeted distinct subpopulations of rhythmic neurons for visualisation using the juxtacellular method.
- tested the synaptic target specificity of labelled medial septal neurons.

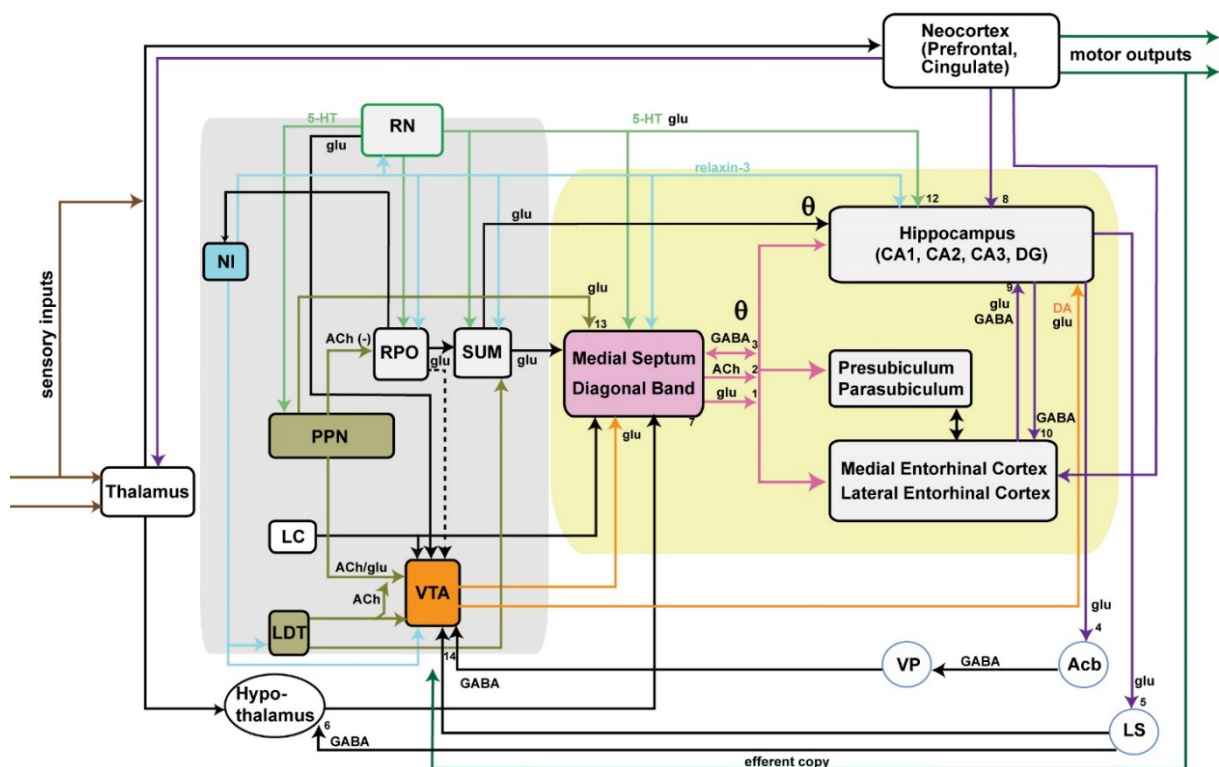


Figure 1.1: The medial septal nucleus and theta oscillations

The medial septum acts as a relay junction which transmits subcortical theta related inputs to the hippocampal formation and extrahippocampal cortical areas. Orange lines: dopamine projections; light green lines: serotonin projections; blue lines: relaxin-3 projections from the

nucleus incertus; pink lines: medial septal projections to the hippocampal formation and extrahippocampal cortices; green olive lines: glutamate and acetylcholine co-projections; the dashed line: sparse GLU connections. black lines: other subcortical projections; purple line: cortical projections. Light grey box: brainstem-hypothalamic network. Dark yellow box: medial septal relay of theta inputs to the hippocampus and extrahippocampal cortices. **Abbreviations:** Acb, nucleus accumbens; DBB, diagonal band of Broca; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; LS, lateral septum; NI, nucleus incertus; PPN, pedunculopontine nucleus; RN, raphe nuclei; RPO, nucleus pontis oralis; SUM, supramammillary nucleus; VP, ventral pallidum.

The theta diagram presented here is modified from Orzel-Gryglewska et al., 2015.

My additions to the scheme are based on the following studies:

1. Medial septal glutamatergic projection to hippocampus and extrahippocampal cortices (Colom et al., 2005; Fuhrmann et al., 2015)
2. Medial septal cholinergic projection to the hippocampus and extrahippocampal cortices (Frotscher and Léránth, 1985; Gaykema et al., 1990)
3. Medial septal GABAergic projection to the hippocampus and extrahippocampal cortices (Freund and Antal, 1988; Gonzalez-Sulser et al., 2014; Unal et al., 2015)
4. Hippocampal glutamatergic projections to the nucleus accumbens (Floresco et al., 2001)
5. Hippocampal glutamatergic projection to the lateral septum (Sweeney and Yang, 2015)
6. Lateral septal projections to the hypothalamus (Risold and Swanson, 1997)
7. Hypothalamic projections to the lateral (Risold and Swanson, 1997) and the medial septum (Fuhrmann et al., 2015)
8. Direct prefrontal cortical projection to the hippocampal formation (Rajasethupathy et al., 2015)
9. Entorhinal cortical projections (glutamatergic and GABAergic) to hippocampal formation (Basu et al., 2016; Kitamura et al., 2014; Melzer et al., 2012; Witter et al., 1988)
10. Hippocampal GABAergic projections to entorhinal cortex (Melzer et al., 2012)
11. Thalamocortical connection
12. Glutamatergic projection to the hippocampus from median raphe (Varga et al., 2009)
13. Glutamatergic projection from the mesencephalic locomotor region to basal forebrain (Lee et al., 2014)
14. GABAergic projection from lateral septum to the VTA (Luo et al., 2011)

Chapter 2: Experimental procedures and analytical methods

2.1 Experimental subjects

All procedures involving experimental animals were under approved personal and project licenses in accordance with the Animals (Scientific Procedures) Act, 1986 (UK) and associated regulations under approved project licence (30/3240) at the University of Oxford. I performed extracellular electrophysiological recordings in adult male C57Bl6/J mice (Charles River, UK) using either multichannel silicon probes (n = 11 mice) or glass electrodes (n = 31 mice). At the time of surgery, mice ranged between 3 and 6 months of age. Mice were housed with littermates until surgical implantation of the head-plate, after which they were housed individually and maintained on a 12 hour light/dark schedule with lights off at 7:00 pm. All behavioural training and recording occurred in the light phase. I also used histological sections from the brains of 6 control mice for immunohistochemistry fixed in an identical way as the experimental mice (see below).

2.2 Surgery

For surgical implantation, mice were deeply anaesthetised with isoflurane (IsoFlo, Abbott Laboratories Ltd, UK) using an induction chamber (3% - 4% v/v with airflow) and given a subcutaneous injection of buprenorphine (Vetergesic, 0.08 mg/kg). Anaesthesia was confirmed by detecting deep breathing, slow heart rate and lack of foot pinch reflex. The mouse was head-fixed in a stereotaxic frame (David Kopf Instruments, CA) via ear bars and kept on a heating pad set to keep rectal temperature at 37°C (homeothermic blanket control unit; Harvard Apparatus, MA). The skull was exposed under aseptic conditions, and three sites were marked: the bregma, medial septal craniotomy (AP: +0.86 mm, ML: 0 mm, DV: 3.5 mm, 0°), and hippocampal craniotomy (AP: -2.4 mm, ML: 1.7 mm, DV: 1.2 mm, 10° postero-anterior angle).

The head-plate (either a 0.7g or 1.1g version, custom made at the Department of Physics, Oxford University) used for head-fixation was secured to the skull using dental cement (Refobacin R, Biomet) and three small M1 screws. Two such screws were fixed to the skull above the cerebellum and served as the ground and electrical reference for the recordings.

2.3 Behavioural procedures

Upon recovery from the surgery (typically 3 to 4 days), mice were trained to run on an air-flow suspended styrofoam ball (Jetball, PhenoSys, for multichannel silicon probes) or spherical treadmill (for single cell recordings). After recovery, food restriction (to maximum 90% of initial pre-surgery body weight) was aimed to motivate running and mice received small drops of sucrose (20% solution) reward upon reaching the end of the linear maze on the styrofoam ball. Mice that performed anticipatory licking and whose tail was balanced, as if running on a linear track, were considered trained. This training was an attempt to standardise behaviour amongst animals, and typically took 7 to 14 days for each animal. Trained mice exhibited a variety of natural behaviours, such as grooming, locomotion, whisking, sniffing, and yawning under head-fixation as they do in the home cage (Video 2.1).

2.4 Recordings and single unit identification

The recording sessions lasted between 30 to 60 minutes in case of multi-channel silicon probe recordings and up to 3 hours for single cell recordings while aiming for neurons with a particular firing pattern. The activity of medial septal cells was recorded during multiple running (RUN) and immobility (REST) periods. Local field potential (LFP) theta oscillations were recorded in the pyramidal cell layer or in stratum oriens in CA1 and referenced to a screw in contact with the dura above the cerebellum, with 0° set as the trough. I identified the recording location by positive going ‘sharp waves’ in the LFP during REST, which were recognised by the co-occurrence of 130-230 Hz filtered ‘ripple’ oscillations. Sharp waves appear as a negative

potential in stratum radiatum, and as positive wave in strata oriens and pyramidale. Wide-band (0.1 - 6000 Hz; 20 kHz sampling rate) recordings were performed using a 2 shank acute silicon probe (150 μ m inter-shank distance; 2 tetrodes per shank; 25 μ m spacing between contacts within a tetrode, Neuronexus) connected to a RA16-AC preamplifier (Tucker-Davies). Recordings were then digitally high pass filtered (0.8 – 5 kHz) and neuronal spikes were detected using a threshold crossing based algorithm. Detected spikes were automatically sorted using the algorithm implemented in KlustaKwik (Kadir et al., 2014), followed by manual adjustment of the clusters (Csicsvari et al., 1999) to obtain well-isolated single units, based on cross-correlations, spike-waveform, and refractory periods. Multiunit or noise clusters or those with less than 300 spikes were discarded from further analysis.

2.5 Juxtacellular labelling

Extracellular single-cell recording and juxtacellular labelling with neurobiotin reveals the identity of the labelled cells in conjunction with their *in vivo* activity patterns, target area and synaptic targets, if the cell is fully labelled, which is difficult to achieve. Using multi-unit recordings in awake head-fixed mice, it was possible to identify the stereotyped activity patterns of Teevra neurons. Spikes of putative Teevra, Komal, and other neurons were recorded during RUN and REST periods (Video 3.2 and Video 4.1) using an extracellular glass electrode filled with 3% neurobiotin in 0.5 M NaCl and the spike output was converted into an audio signal. First the target cell was recorded from a distance (estimated by the amplitude of spikes recorded by a glass electrode with known impedance) to ensure that multiple RUN and REST periods could be recorded without damaging the cell. Once the activity of the target cell was recorded reliably and if the neuron was deemed to be a Teevra cell, the glass electrode was advanced towards the cell using a motorised stepper (Scientifica IVM single micromanipulator). Positive pulses of current (few nA separated by 250 ms) were injected next to the cell to modulate the firing of the cell for at least 30 seconds. The potential success of juxtacellular labelling was

predicted by analysing cellular health post-labelling *in vivo*, by comparing the spike waveform and firing pattern before and after the modulation attempt. Successful labelling attempts were defined as those in which the cell was modulated for more than 30 sec and action potentials could still be seen and heard after the cessation of the modulation attempt. In such cases, the animal was perfused after 4 to 8 hours to allow for the transport of Neurobiotin and potential terminal bouton labelling, which was assessed after processing the brain. In addition to the most numerous *Teevra* ('sharp sound of burst') cells, the second group, which I named *Komal* ('soft sound of burst') could be reliably differentiated from *Teevra* cells based on their long burst duration and increase in firing rate during RUN (Video 3.2 and Video 7.1). Overall, during my studies, I have recorded 317 septal neurons using glass electrode recordings and made 20 labelling attempts. I included those cells in the study which have (a) multiple running and resting periods and (b) in which the location of the glass electrode could be established in the medial septum, either by virtue of its proximity to a labelled cell or a previously recorded rhythmic firing neuron. Some labelled cells could not be analysed and included here since I concentrated on *Teevra* and *Komal* cells due to the duration of my studies.

2.6 Tissue processing and immunohistochemistry

Mice were deeply anaesthetised with sodium pentobarbital (50 mg/kg, i.p.) and transcardially perfused with saline followed by a fixative solution (4% paraformaldehyde, 15% v/v saturated picric acid, 0.05% glutaraldehyde in 0.1 M PB at pH 7.4). This fixative is optimised for light and electron microscopic immunohistochemistry preserving most, but not all, antigenic epitopes (Somogyi and Takagi, 1982). Some brains were post-fixed overnight in glutaraldehyde-free fixative. After washing in 0.1 M PB, coronal sections (70-80 μ m thickness) were cut using a vibratome (Leica VT 1000S, Leica Microsystems) and stored in 0.1M PB with 0.05% sodium azide at 4°C for further processing. The neurobiotin-labelled processes could be visualised in selected sets of individual sections using streptavidin-conjugated fluorophores

following permeabilisation by Tris-buffered saline (TBS) with 0.3% Triton X-100 (TBS-Tx) or through rapid 2x freeze-thaw (FT) over liquid nitrogen (cryoprotected in 20% sucrose in 0.1 M PB). To visualise proteins within selected labelled cellular domains or in their synaptic targets, the sections were first blocked for 1 hour at room temperature (RT) in 20% normal horse serum (NHS) to reduce non-specific binding of antibodies. Then they were incubated in primary antibody solution containing 1% NHS for 2 to 4 days at 4°C. For the specificity of the method, negative controls lacked the primary antibodies and they were processed in parallel with the sections containing the labelled neurons. Primary antibodies (Table 2.1) were detected with fluorophore-conjugated secondary antibodies for wide-field epifluorescence and confocal microscopy (Ferraguti et al., 2004; Lasztóczy et al., 2011). After primary antibody incubation, sections were washed three times for 10 min and transferred to a secondary antibody solution containing 1% NHS for 4 hours at RT or overnight at 4°C. Following secondary antibody incubation, sections were washed three times for 10 min each and mounted on glass slides in VectaShield. In each incubation, up to 4 antibodies raised to detect different molecules were used. This strategy exploits the distinct subcellular locations of target proteins (e.g nucleus, plasma membrane). Using this approach, the labelled process or synaptic target neurons could be simultaneously processed with 4 primary antibodies, which could be repeated subsequently multiple times if the cellular domain to be tested lacked detectable immunoreactivity in the previous round of reactions or if the molecule was not in the same cellular compartment. I was able to test the same cellular profile in a given section for up to 6 different molecules and different sections for up to 8 molecules of a single cell.

For light microscopic visualisation, the neurobiotin signal was amplified by incubating the TBS-Tx or FT processed sections first in biotin (component B in VECTASTAIN Elite ABC HRP Kit) then in avidin-biotin complex (VECTASTAIN Elite ABC HRP Kit) for 3 to 7 days depending on the strength of labelling. Incubating for longer duration sometimes improved the

neurobiotin visualisation, but also increased endogenous biotin detection. The sections were then processed using horseradish peroxidase (HRP) based diaminobenzidine (DAB) reactions, using the glucose oxidase method for the generation of H₂O₂ as substrate and nickel-intensified DAB as chromogen (Shu et al., 1988). The sections were osmium tetroxide treated (0.5-1% in 0.1 M PB) in order to amplify signal intensity, sequentially dehydrated, and mounted on slides in epoxy resin (Unal et al 2015). Selected sections for electron microscopy were incubated in 2% wt/vol uranyl acetate during dehydration, in order to increase the electron opacity of cellular membranes.

2.7 Light and florescence microscopy

Wide field epifluorescence microscopy and confocal microscopy (Carl Zeiss LSM 710) were used to evaluate immunohistochemically reacted sections. Sections were first observed with a wide field epifluorescence microscope (Leitz DMRB; Leica Microsystems) equipped with PL Fluotar objectives. Multichannel fluorescence images were acquired with ZEN 2008 software v5.0 on a Zeiss LSM 710 laser scanning confocal microscope (Zeiss Microscopy), equipped with DIC M27 Plan-Apochromat 40/1.3 numerical aperture, DIC M27 Plan Apochromat 63/1.4 numerical aperture, and Plan-Apochromat 100/1.46 numerical aperture oil-immersion objectives. The following channel specifications were used (laser/excitation wavelength, beam splitter, emission spectral filter) for detection of Alexa405, Alexa488/EYFP, Cy3, and Cy5: 405-30 solid-state 405 nm with attenuation filter ND04, MBS-405, 409–499 nm; argon 488 nm, MBS-488, 493–542 nm; HeNe 543 nm, MBS-458/543, 552–639 nm; HeNe 633 nm, MBS-488/543/633, 637–757 nm. The pinhole size was set to 1 Airy unit for the shortest wavelength and, to keep all channels at the same optical slice thickness, the pinhole sizes of the longer wavelength channels were adjusted to values close to 1 Airy unit. Thus, optical section thickness for all channels was based on the set pinhole size for the shortest wavelength channel.

The osmium-treated sections mounted in resin after the HRP enzyme reaction (DAB) were analysed using transmitted light microscope. To reveal the axonal arbour of Teevra cells in the CA3 and sample boutons in different regions and layers, two-dimensional reconstructions were made with a drawing tube attached to the transmitted light microscope equipped with a 63X oil-immersion objective. For all visualised axons, the first and last antero-posterior section containing boutons and the most caudal section with the axon were established.

2.8 Electron microscopy (performed by Prof Peter Somogyi)

The correspondence of light microscopically predicted putative synapses between target cells and axonal varicosities of Teevra cells was tested by electron microscopy. I selected axon collaterals in osmium-treated sections. This test enabled us to determine the probability of determining synaptic junctions based on axonal swellings next to target profiles. Serial sections (70 nm) were cut and mounted on single-slot, pioloform-coated copper grids for conventional transmission electron microscopy. Images were acquired with a Gatan UltraScan 1000 CCD camera. All neurobiotin containing boutons cut at the section plane were followed in serial sections to locate synaptic junctions. Synapses were identified as Gray's type I (often called asymmetrical) and type II (often called symmetrical) based on their fine structure; type I synapses have a thick postsynaptic density, whereas type II synapses are characterized by a thin postsynaptic density (Gray, 1959). I participated in each phase of this test and in the interpretation of images, but due to the duration of my scholarship, I did not have time for training to become independent in electron microscopy. I received tutorials in the preparation of such specimens and did so independently.

2.9 Electrophysiological data analysis

Data were analysed in MATLAB (R2014a, MathWorks) and Spike2 (CED). RUN was defined as locomotion, whereby the jetball (in virtual reality) or circular treadmill (for single cell experiments, Table 4.1) was physically advanced by the mouse, based on virtual reality

feedback or video observations. For single cell experiments, this additionally included signals from a motion sensor within the circular treadmill. The initiation of locomotion was also included in RUN. Small postural shifts in the absence of limb motion were excluded from RUN. For labelled Teevra cells MS71c, MS73c, and MS90g, in addition to video analysis, signals from an external accelerometer in contact with a running disc were used to define RUN. REST periods included everything outside RUN periods.

RUN periods: I analysed the hippocampal LFP using algorithms written by Dr. John Tukker. First the LFP was digitally band pass filtered (finite impulse response, FIR, Gaussian window, 1024/2048 coefficients, transition gap 2.5 Hz) at delta (2 to 4 Hz) and theta frequencies. Theta periods were selected based on the theta to delta frequency power ratio using Fast-Fourier transforms (FFT, 1024 points, 2 s Hanning windows). A ratio greater than four in three consecutive windows was identified as a theta epoch. Theta cycles were defined between two consecutive troughs during the selected periods. In my studies, all RUN periods in trained animals consistently displayed high power theta oscillatory activity in the 7 to 9 Hz frequency range.

REST periods: In the absence of movement related theta oscillations, large amplitude irregular activity (LIA) was prominent in the hippocampal LFP during immobility epochs which I defined as REST. There was no or little contribution by sleep as the animals were mostly awake and alert even during REST periods. To detect the zero crossings of waves in LIA, the CA1 reference LFP channel was down sampled to 1 kHz, the DC component was removed from the signal using a time constant of 0.08 sec, and the zero crossings were detected at falling or rising levels with minimum interval of 200 ms between consecutive crossings. For detecting SWRs, the threshold was set to power values (r.m.s in 10 ms sliding windows) of 5x s.d. above the mean power. The start and end of ripples were set to time points where the r.m.s crossed 1x s.d.

above the mean power. To determine if the activity patterns of a given neuron are dependent on SWRs, I have used algorithms developed by Dr. Linda Katona (Katona et al., 2014).

2.10 Rhythmicity Index

Rhythmic burst firing is a characteristic feature of medial septal neurons. For all recorded neurons, I have calculated the Rhythmicity index, the calculation of which is based on the ‘theta index’ (Royer et al., 2010), but it has fewer parameters (3 vs 6 in Royer et al., 2010), for a more robust fit. The RI was developed by Dr Robert Stewart. Data were prepared by calculating the spike time autocorrelogram (bin width 10 ms, maximum lag 500 ms) for spikes defined in periods of RUN or REST. Next, the autocorrelogram was normalised by dividing by the peak value between 100 and 200 ms (range chosen to match theta frequency first side band), and centre values were clipped so that overall maximum is one. A linear trend line was fitted to the normalised autocorrelation histogram, and a non-linear fit is applied (using MATLAB `lsqnonlin` function) to the detrended data. The fitting function is a Gaussian-modulated cosine function with three parameters: (1) cosine (theta) frequency in Hz (between 4 and 8); (2) the peak value of the Gaussian scaling function (high value indicates strong short-latency theta modulation) and (3) standard deviation (width) of Gaussian scaling function (high value indicates prolonged theta modulation). The solid red lines in Fig. 3.2, Fig. 3.4, Fig. 4.1, Fig. 5.1, and Fig. 6.1 are the fitted sinusoid functions, which represent the oscillatory activity of burst firing for the neuron. The trends are shown by dotted lines. A coefficient of determination is measured at this stage to measure the goodness of fit. After fitting, the rhythmicity index is calculated as follows: (1) for each peak and trough in 50 to 500 ms, the absolute value of the fitted sinusoid is divided by the corresponding trend line value (values between zero and one) and (2) the rhythmicity index is taken as the mean of these trend-normalized peak values.

2.11 Burst duration

I have calculated the bursts of medial septal action potentials using an algorithm in which spikes were gathered by growing groups such that nearest neighbour spikes were iteratively added unless interspike interval (ISI) was greater than the threshold. When both neighbouring ISIs are greater than the threshold, the burst was delimited: min spikes = 2, threshold = mean ISI. This parameter reliably identified groups of spikes which could be identified as bursts during RUN periods dominated by theta epochs (Fig. 3.4 and Fig. 3.6). Mean burst durations during RUN are reported throughout the thesis.

2.12 Rate change score and speed correlation

Mean firing rates (Hz) were calculated during RUN and REST periods. A rate change score was then computed according to the following formula:

$$\text{rate change score} = \frac{\text{rate(RUN)} - \text{rate(REST)}}{\text{rate(RUN)} + \text{rate(REST)}}$$

Score ranges from -1 (decrease of rate during running) to 1 (increase of rate during running). The speed modulation of MS unit firing was also calculated for each neuron recorded using multi-channel probes. Data was binned at 1 cm/s. For each speed bin (>2cm/s), the corresponding firing frequency was calculated as the number of detected action potentials while the mouse was moving at this speed, divided by the duration the mouse ran at this speed. The firing frequencies per speed bin were linearly fitted with a weighting function for each bin equal to the square root of its duration. A linear correlation coefficient was computed for each neuron and it was determined if the firing is positively modulated ($r>0$, $p<0.05$), negatively modulated ($r<0$, $p<0.05$), or not correlated with the running speed of the animal ($p>0.05$).

2.13 Mean firing phase preference

To determine the mean firing phase preference for each recorded neuron, theta cycles were detected and spikes were sorted into 18° theta-phase bins, cycle-by-cycle. The phase corresponding to every spike time (t_s) was computed between the preceding (t_i) and following (t_{i+1}) theta trough times using:

$$\text{phase}_s = [(t_s - t_i)/(t_{i+1} - t_i)] \times 360^\circ$$

Thus each spike was assigned an instantaneous phase between two troughs (0° and 360°). The spike-timing relative to the theta cycles in CA1 stratum pyramidale or oriens was plotted on polar phase histograms (Fig. 3.4, Fig. 4.1, and Fig. 7.3). For each recorded neuron, the mean depth of theta modulation and preferential mean theta phase of firing (circular mean $\pm$ circular SD) was determined using Rayleigh's method (Zar, 1999).

Group differences between the preferential mean theta phase of firing for two clusters of rhythmic medial septal cells was determined using Watson's- U^2 test.

2.14 Hierarchical clustering

All analyses were performed using 'Cluster Analysis' toolbox in MATLAB (R2014a, MathWorks). All parameters (m) for all neurons (n) are prepared in an 'm-by-n' data matrix. The data is linearly rescaled and set to a new defined min and max (-1 to 1, rescale function, MATLAB) and stored in data matrix (e.g. X). Next, I calculate the pairwise Euclidian distance between each pair of observations in X (using MATLAB 'pdist' function) and store this in a matrix (e.g. D). Next, I calculate the linkages and create a hierarchical cluster tree. MATLAB function linkage returns a tree (e.g. Z) that encodes hierarchical clusters of the real matrix X using 'Ward' method to measure the distance between clusters. A tree encoded by Z is plotted (using MATLAB function 'dendrogram') and I identified four major branches (using MATLAB function 'cluster') and the smallest height at which a horizontal line cut through the

tree (using MATLAB function ‘maxclust’). The cluster value assignment for each neuron is stored in a matrix (e.g. C). The silhouette method is used to determine the goodness of clustering. The silhouette value (MATLAB, Cluster Analysis toolbox, range: -1 to 1) for each point is a measure of how similar that point is to points in its own cluster vs. points in other clusters. Silhouette value for a cluster is reported as the mean silhouette of its individual members.

2.15 Quantification and statistical analysis

Standard functions and custom-made scripts in MATLAB were used to perform all analysis. I have not estimated the minimal population sample for statistical power, but the number of animals and labelled neurons were similar to or larger than those employed in previous works (Katona et al., 2014; Pernía-Andrade and Jonas, 2014; Tukker et al., 2015; Varga et al., 2014; Viney et al., 2013). The data were tested for normal distribution. Parametric tests were used for normally distributed data and non-parametric tests were applied to all other data. For each recorded neuron, the mean depth of theta modulation and preferential mean theta phase of firing (circular mean $\pm$ circular SD) was determined using Rayleigh’s method (Zar, 1999). For comparisons of firing phase preferences of different cell types, I used Watson’s U² test. Kruskal-Wallis one-way analysis of variance was used to compare two groups. Box-plots represent median and 25th – 75th percentiles and their whiskers show data range. Outliers are shown as a ‘+’ sign and were included in all analysis.

Table 2.1: Reagents and resources used

Antibodies to			
REAGENT	SOURCE	IDENTIFIER	SPECIFICITY INFORMATION
Calbindin (CB) [host Rb; Dil 1:5000]	Swant	Cat. No. CB 38; RRID:AB_10000340	(Airaksinen et al., 1997) – knock out test
Calretinin (CR) [host Rb; Dil 1:500]	Swant	Cat. No. 7699/3H; RRID:AB_10000321	Labelling pattern as published with other antibodies
Metabotropic glutamate receptor 1a (mGluR1a) [host Gp; Dil 1:1000]	Frontier Institute	Cat. No. mGluR1a-GP-Af660; RRID:AB_2531897	(Nakamura et al., 2004; Tanaka et al., 2000)
Parvalbumin (PV) [host Rb; Dil 1:1000]	Swant	Cat. No. PV 28; RRID:AB_2315235	(Kagi et al., 1987; Schwaller et al., 1999) – knock out test
Parvalbumin (PV) [host Gt; Dil 1:1000]	Swant	Cat. No. PVG 214; RRID:AB_10000345	No signal in KO animal (Swant), similar labelling as previously published antibodies
Parvalbumin (PV) [host Gp; Dil 1:5000]	Synaptic Systems	Cat. No. 195 004; RRID:AB_2156476	Labelling pattern as previously published antibodies
Pro-cholecystokinin (pro-CCK) [host Rb; Dil 1:500]	April 2005 gift (similar to Frontier Institute, CCK-pro-Rb-Af350)		Similar labelling to two non-commercial antibodies characterized by Sloviter and Nilaver (<i>The Journal of Comparative Neurology</i> , 1987, 256, 42–102), Morino et al. (<i>The European Journal of Neuroscience</i> , 1994, 6, 681–773)
SATB1 (N-14) [host Rb; Dil 1:200]	Abcam	Cat. No. ab70004; RRID:AB_1270545	No signal in knock out. Huang et al. <i>Neuroscience Research</i> , 2011, 71, 12-33 and Balamotis et al. <i>Molecular and Cellular Biology</i> , 2012, 32, 333-380
SATB1 (N-14) [host Gt; Dil 1:200]	Santa Cruz	Cat. No. sc-5989; RRID:AB_2184337	Similar to the rabbit antibody ab70004
Somatostatin (SST) [host Mo; Dil 1:500]	GeneTex	Cat. No. GTX71935; RRID:AB_383280	Labelling pattern similar to the rat antibody Chemicon MAB364 (Kubota et al., 2011)
Somatostatin (SST) [host Rb; Dil 1:50]	GenWay Biotech	Cat. No. 18-783-76392; RRID:AB_1027453	Labelling pattern similar to previously published
Vesicular GABA transporter (VGAT) [host Rb; Dil 1:500]	Synaptic Systems	Cat. No. 131 003; RRID:AB_887869	Western blot; band at 57kDa. Similar to guinea pig antibody. Characterisation: Fukudome et al., <i>The European Journal of Neuroscience</i> , 2004, 19, 2682 - 2774
Vesicular GABA transporter (VGAT) [host Gp; Dil 1:500]	Synaptic Systems	Cat. No. 131 004; RRID:AB_887873	Same epitope and production as Takamori et al. <i>J. Neurosci.</i> , 2000, 20, 4904 - 4915
Vesicular Glutamate transporter (VGluT2) [host Gp; Dil 1:2000]	Synaptic Systems	Cat. No. 135 404; RRID:AB_887884	Characterized in mouse hippocampus by Herzog et al. 006 <i>J. Neurochem.</i> 99, 1011-1018

Substance P receptor (NK1R) [host Rb; Dil 1:2000]	Millipore	Cat. No. AB5060 RRID:AB_2200636	Characterised by Shigemoto et al. 1993 <i>Neurosci. Lett.</i> 153 , 157-160
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Rb, rabbit; Gt, goat; Mo, mouse; Gp, guinea pig

Chemicals, Peptides, and Recombinant Proteins		
REAGENT	SOURCE	IDENTIFIER
Neurobiotin Tracer	Vector Laboratories	Cat. No. SP-1120
Critical Commercial Assays		
REAGENT	SOURCE	IDENTIFIER
Vectastain ABC kit	Vector Laboratories	Cat. No. 6100
VECTASHIELD Antifade Mounting Medium	Vector Laboratories	Cat. No. H-1000
Experimental Organisms/Strains		
RESOURCE	SOURCE	IDENTIFIER
C57Bl/6J mouse	Charles River	JAX Mice Stock Number 000664
Software and Algorithms		
RESOURCE	SOURCE	IDENTIFIER
MATLAB	MathWorks	Version R2014a; https://uk.mathworks.com/products/matlab.html
Spike2	Cambridge Electronic Design Limited (CED)	Spike2 version 7 and version 8; http://ced.co.uk/
KlustaKwik	Harris et al., 2000	http://klustakwik.sourceforge.net/
Zen	Zeiss	Version 2.1; https://www.zeiss.co.uk/corporate/home.html
Fiji	ImageJ	(Schindelin et al., 2012)
Scalable Brain Atlas	Allen Brain Atlas	(Bakker et al., 2015)
Other		
RESOURCE	SOURCE	IDENTIFIER
Silicon probe; A2x2tet-10mm-150-150-121	NeuroNexus	http://neuronexus.com/
Jetball Dome	PhenoSys	http://www.phenosys.com/products/virtual-reality/jetball-dome/

Chapter 3: Identification of medial septal neuron populations based on spike train dynamics: Teevra and Komal neurons

3.1 Introduction

The destruction of the medial septum or hippocampal fimbria, or the inactivation of the septum by pharmacological manipulation results in loss of theta oscillatory power and impaired performance in spatial learning (Winson, 1978; Chrobak et al., 1989; Brioni et al., 1990), disruption of learning in contextual fear conditioning (Calandreau et al., 2007), impairment in working memory (Givens and Olton, 1994) and slows the rate of acquiring delayed eyeblink conditioning (Berry and Thompson, 1979). All these memory-guided behaviours depend on the hippocampus and their disruption suggests that the medial septal input plays a crucial role in coordinating the hippocampal theta rhythm and associated mnemonic processes. The medial septum sends axonal projections to the hippocampus via GABAergic (Freund and Antal, 1988; Gaykema et al., 1990), glutamatergic (Fuhrmann et al., 2015; Sotty et al., 2003), and cholinergic projections (Gielow and Zaborszky, 2017), however the cell type specific mechanisms through which the septum coordinates hippocampal theta oscillatory activity are unknown. Under anaesthesia, the medial septal GABAergic neuronal population fire rhythmic bursts of action potentials in the 5 - 12 Hz theta frequency range and have been hypothesised to be a pacemaker of the hippocampal theta oscillation (Borhegyi et al., 2004a; Gogolák et al., 1968; Petsche et al., 1962; Simon et al., 2006). In such a preparation, cholinergic neurons are slow firing and the activity pattern of glutamatergic neurons, which form roughly one third of the medial septal cellular population is unknown.

Various neuronal elements cooperate in time for the processing and storage of information and it is known that the targets of medial septal GABAergic neurons are hippocampal and extrahippocampal GABAergic interneurons (Freund and Antal, 1988; Unal et al., 2015). These

target interneurons exhibit a wide diversity in activity patterns during theta, gamma, and ripple oscillations (Katona et al., 2014; Klausberger and Somogyi, 2008; Lasztóczy and Klausberger, 2014; Varga et al., 2012; Viney et al., 2013), but how subcortical inputs might contribute to their diversity is largely unknown. Previous studies in awake rats focused on putative GABAergic rhythmic firing versus putative cholinergic non-rhythmic septal neurons (Dragoi et al., 1999; King et al., 1998). Given the diversity and complexity of the potential post-synaptic targets of rhythmic MS neurons, it is unlikely that there is only one type of GABAergic cell type in the medial septum (Borhegyi et al., 2004). It is plausible that septal GABAergic neurons parallel the cortical interneuron diversity with distinct subpopulations of septal neurons innervating distinct sub-populations of hippocampal GABAergic neurons. To test this hypothesis and to explain the contribution of septal neuronal firing to the coordination of theta oscillations, it is necessary to test the diversity of activity patterns of MS neurons in awake animals since theta oscillatory activity in rodents is linked to voluntary locomotor activity (Vanderwolf, 1969). Therefore, in the following chapter, I explore the diverse activity patterns of medial septal neurons in awake behaving mice.

I have performed both extracellular multi-unit and single cell recordings in head-fixed mice with the aim of identifying and classifying the GABAergic subpopulation of medial septal neurons. Classification of neurons is a major challenge in systems neuroscience and there is no consensus on how to define a cell type. One view is that “*Two individual neurons belong to the same cell type if they deliver the same neuroactive substances to the same range of postsynaptic targets in the same temporal patterns in a brain state-specific manner*” (Somogyi, 2010). I will attempt to apply this definition to the medial septal neurons in this thesis. In this chapter, I explore the diversity in septal neuronal firing and introduce the use of unsupervised hierarchical cluster analysis to identify septal neuronal subpopulations based on spike-train dynamics.

3.2 Experimental setup

Two experimental approaches were used to monitor the activity patterns of medial septal neurons. Mice were either trained to run on an air-flow suspended styrofoam ball (for multichannel silicon probe recordings, $n = 11$ mice, Video 3.1) or a spherical treadmill (for single cell recordings, $n = 31$ mice). The recording locations were established by painting the silicon probe with DiI (Vybrant Multicolour Cell-Labeling Kit, Invitrogen) and only those experiments were included in which the recording location could be correctly identified ($n = 7$ experiments tetrode recordings). Food restriction (to minimum 90% of initial body weight) was used to promote motivated running behaviour by the provision of small drops of sucrose reward during recordings. The mice were trained for 7 to 14 days to navigate on the virtual linear maze or acclimatise to the spherical treadmill. Dorsal CA1 stratum pyramidale LFP activity was recorded simultaneously and served as a reference across experiments. I used hierarchical cluster analysis to cluster septal neurons using calculated physiological parameters as feature vectors. Theta phase firing preference was used as an additional, independent feature to cross-validate the goodness of clustering.

3.3 Medial septal neurons exhibit diverse firing rates and firing phase preferences with respect to hippocampal theta oscillations

Using multichannel extracellular probes, the neuronal activity in the septum of head-fixed mice ($n = 7$) navigating on a virtual linear maze was examined. Mice alternated between running (RUN) and immobility (REST) epochs during this task. The location of the probe and recording sites were established histologically in fixed brain sections post hoc, and further analysis was restricted to the cases where several recording sites were confirmed to be in the medial septum (MS) ($n = 4$ mice, Fig. 3.1). The other animals had recording sites in the adjacent lateral septum (LS), which surrounds the MS from all sides. The action potential firing frequency of MS recorded neurons during running varied widely (median: 23.98 Hz; inter quartile range (IQR):

13.4 - 38.5 Hz, $n = 81$ neurons) and was higher than that of adjacent lateral septal (LS) neurons (median: 2.55 Hz, IQR: 1 – 7.1 Hz, $n = 18$ neurons; Kruskal-Wallis test, $p < 10^{-8}$). All MS neurons recorded in this configuration were phase coupled to ongoing theta oscillations recorded in dorsal CA1, whereas this was only true for 27% of lateral septal neurons (Rayleigh test, $p = 0.05$ significance level). Hence, medial septal neurons could be reliably differentiated from adjacent lateral septal neurons using firing rate during locomotion and phase coupling to ongoing hippocampal theta oscillations. These two features were used subsequently to identify medial septal neurons based on extracellularly recorded spikes.

Simultaneously recorded medial septal neurons ($n = 16$) exhibited a high variation in firing rates and their firing phase preferences covered the entire theta cycle as recorded in the pyramidal layer of dorsal CA1 (Fig. 3.1A). At a population level, MS neurons ($n = 81$) showed a slight preference to theta troughs (Fig. 3.1B).

These results provide insight into the activity patterns of simultaneously recorded septal neurons and also provide a framework to distinguish between MS and LS neurons.

3.4 Diverse rhythmicity and burst duration

Rhythmic burst firing is considered to be a characteristic feature of MS GABAergic neurons (Petsche et al., 1962; Gaztelu & Buno 1982; Alonso et al., 1987., King et al., 1998; Ford et al., 1989; Dragoi et al., 1999; Simon et al., 2006). However, in my recording data set, I observed a diversity in the extent of rhythmicity of action potential (AP) firing based on the autocorrelation histograms (Fig. 3.2A). To capture this, a rhythmicity index (RI, developed by Dr. Robert Stewart, Chapter 2.10) was calculated for each neuron. The RI bounded from 0 to 1 in order of increasing rhythmicity. Briefly, a Gaussian modulated cosine function was fitted on the detrended autocorrelation histogram and the absolute value of the fitted sinusoid is divided by the corresponding trend line value for each peak and trough. The rhythmicity index is based on

‘theta index’ (Royer et al., 2010) but uses fewer parameters (3 instead of 6) for a more robust fit. This index provided a parameter which was independent of the theta frequency range and reliably captured the variation in rhythmicity of individual neurons (Fig. 3.2A). I also estimated the mean burst duration to capture the diversity in the action potential firing. Briefly, spikes were added by growing groups such that nearest neighbour spikes were iteratively added unless interspike interval (ISI) was greater than the threshold (see Chapter 2.11).

During RUN periods, simultaneously recorded MS neurons (range: 5 to 19) exhibited varying burst duration (median: 55 ms, IQR: 40.6 – 87.9 ms, $n = 81$ neurons) and extent of rhythmicity (median: 0.13, IQR: 0.04 – 0.31, $n = 81$ neurons). At a population level, I observed that a rhythmicity index cut-off of 0.1 reliably differentiated between neurons whose autocorrelation histograms showed rhythmicity. I have used this as a cut-off for rhythmic and apparently ‘non-rhythmic’ groups in this study (Fig. 3.2B).

The firing theta phase preference of MS neurons scaled with rhythmicity index; neurons with higher indices preferred the theta trough.

3.5 Diverse firing rate responses with running speed

As the medial septum is implicated in movement associated theta oscillation (Buzsáki, 2002; Lawson and Bland, 1993; Stewart and Fox, 1990), the relationship of MS neuronal firing to a change in behavioural state from REST to RUN was determined for each neuron. This firing rate change was measured as a score ($\text{Diff RUN} - \text{REST} / \text{Sum}$, Chapter 2.12) which bounded from -1 to 1 (Fig. 3.3A). Within the RUN, MS neurons showed diverse relationship to the running speed of the animal (Fig. 3B). This could be measured by a linear correlation coefficient of the septal neuronal firing with running speed. Individual MS neurons could increase (aj27b-unit9), decrease (aj31b-unit11), or not change (aj27b-unit10) their firing with the running speed

of the animal (Fig. 3.3B). This change was consistent for a given cell in the recording session. Note that each recording session may contain several RUN and REST periods.

3.6 Hierarchical clustering to reveal septal clusters

Because medial septal neurons exhibited a broad diversity in most physiological parameters, I calculated the same parameters for all recorded cells to identify similarities and differences in their activity patterns. An example of the parameters calculated for all neurons is shown in Fig. 3.4. These were the mean burst duration, theta phase firing preference, rhythmicity index (RI), oscillatory frequency of burst firing (OF), firing rate change score, and correlation of the firing with running speed. Similar activity dynamics could be identified and measured in single unit recordings in head-fixed mice on a spherical treadmill ($n = 65$ neurons, $N = 24$ mice) using glass electrodes (Fig. 3.4E). I calculated the same parameters for all MS neurons ($n = 43$ tetrodes, $n = 46$ glass electrodes) recorded using multichannel and single cell extracellular recordings with a rhythmicity index lower cutoff of 0.1 and fed them to an unsupervised hierarchical clustering algorithm using Ward's method (clustering parameters: rate change score, mean burst duration). Subsequently, the characteristics of four major clusters (Fig. 3.5 and Table 3.1) were explored. The goodness of clustering was measured using the mean silhouette value, which ranges from -1 to 1 and indicates cluster cohesion.

The most populous cluster of MS neurons (Group 2; $n = 48$, mean silhouette value: 0.74) was composed of cells that have negligible change in their firing rate from REST to RUN (median rate change score: -0.05, IQR: = -0.14 – 0.005), a high firing rate (median = 30.5 Hz, IQR = 22.8 – 51.4 Hz) and a short burst duration (median: 38 ms, IQR: 33.5 – 42.2 ms, Fig. 3.5). When I converted the burst of action potential waveforms to sound, these neurons had a 'sharp' vocalisation, therefore these are named *Teevra*¹ cells (e.g., neuron aj27b_10 in Fig. 3.6A; Video

¹ When I converted the spikes to sound and listened to them, neurons with a short burst duration have a 'sharp' vocalisation, whereas neurons with a long burst duration sound 'soft' and 'flat' (Video 3.2). This gives them their

3.2). In contrast, MS neurons in the second largest cluster (Group 3; $n = 23$, mean silhouette value: 0.74) increased their firing rate from REST to RUN (median rate change score: 0.21, IQR: 0.16 – 0.29), had a high firing rate (median: 41.5 Hz, IQR: 30.6 – 62.9 Hz) and a long burst duration (median: 57 ms, IQR: 53.4 – 64 ms, Fig. 3.5); these were named *Komal* cells based on the ‘soft’ or ‘flat’ sound of the burst (e.g. neuron aj27b_9 in Fig. 3.6B; see also Video 3.2). Teevra and Komal neurons were different in their burst duration during running ($p = 8.7 \times 10^{-11}$, Kruskal-Wallis test) and in the firing rate change score ($p = 1.2 \times 10^{-11}$, Kruskal-Wallis test), however, their mean firing rates during running periods were not different ($p = 0.12$, Kruskal-Wallis test). The activity of the two groups of neurons recorded using tetrodes also differed in their correlation with running speed, which was measured by a linear correlation coefficient (Teevra cells, median R : -0.02, IQR: -0.14 – 0.11, $n = 21$; Komal cells, median R : 0.37, IQR: 0.21 – 0.53, $n = 12$; $p = 1.5 \times 10^{-5}$, Kruskal-Wallis test), individual examples are shown in Fig. 3.7. In addition to the two largest groups, Group 1 neurons ($n = 4$) decreased firing from REST to RUN and had a low mean firing rate during running (median = 7 Hz, IQR = 4 – 13 Hz), and Group 4 neurons ($n = 14$) increased their firing rate from REST to RUN (median rate change score = 0.27, IQR = 0.18 – 0.32) and had a low firing rate during RUN (median = 14.5 Hz, IQR = 13.2 – 37.1 Hz).

The mean firing phase preference of individual septal neurons with respect to ongoing theta oscillations recorded in dorsal CA1 provides information about possible temporal specialisations in their activity and influence. I tested whether Teevra and Komal neurons were different in the mean firing phase preference relative to CA1 theta. Importantly, this parameter was not used in the clustering previously performed. The pooled firing phase preferences of Teevra and Komal neurons were significantly different (Fig. 3.7; $p < 0.002$, Watson’s U^2 test,

Sanskrit names which are inspired from Hindustani classical music. *Teevra* is the ‘sharp’ vocalisation of the short burst duration neurons, whereas *Komal* is the ‘soft’ or ‘flat’ vocalisation of the long burst duration neurons.

difference of circular means = 160°), with most Teevra neurons firing preferentially around the trough whereas most Komal neurons preferring firing at the peak of dorsal CA1 stratum pyramidale theta LFP. Note that within both groups there are individual neurons with firing phase preferences different from these trends. For Teevra cells the angular phase preference to stratum pyramidale troughs correlated with a higher rhythmicity index (angular-linear correlation coefficient: 0.49, $p = 0.003$, $n = 48$, Fig. 3.7).

3.7 Discussion

The MS and LS are parts of distinct but related hippocampal subcortical circuits. Whereas the MS projects to the hippocampal formation (Gaykema et al., 1990) and receives GABAergic innervation from hippocampo-septal cells (Tóth et al., 1993), the LS receives a major input from the CA1 and CA3 pyramidal cells (Sweeney and Yang, 2015). These LS GABAergic neurons (>90% of LS population) then project to other subcortical structures such as the ventral tegmental area (VTA) and lateral hypothalamus (LH), but probably not to the MS (Luo et al., 2011; Risold and Swanson, 1997; Swanson and Cowan, 1979). Identification of the recording locations based on DiI-labelling and correlating the activity patterns and phase preference with theta oscillations was therefore crucial to attribute the diversity of recorded neurons to the MS.

In this study, I have provided a detailed quantitative characterisation of neuronal populations with single cell resolution and observed diverse firing rates, firing phase preferences, rhythmicity, burst duration, interspike intervals, and speed correlation for the rhythmic population of MS neurons. These observations were made during well-defined behavioural (REST and RUN) and network (theta) states. In the current experiments, the electrophysiological reference locations were aimed at dorsal CA1 stratum pyramidale to obtain a reference that is reproducible across the current study and comparable to previous studies.

In my search for distinct neuronal populations, an unsupervised hierarchical clustering algorithm using the Ward method to calculate Euclidian distances was used to cluster MS rhythmic neurons. The ‘rate change score’ and ‘mean burst duration’ were used as parameters to feed into the hierarchical clustering algorithm. The analysis was restricted to 4 major branches in order to study broad differences between subgroups. There is no universally accepted method of clustering and given any two parameters, the clustering algorithm would always produce a dendrogram, so I chose to test the validity of clustering using an independent parameter not used in the clustering – the firing phase with respect to ongoing theta oscillations in the hippocampus. Teevra and Komal cells exhibited distinct phase preference with respect to ongoing theta oscillations with Teevra cells preferring the peak and Komal cells preferring the trough with respect to dorsal CA1 theta oscillations. Teevra cells may have a significant status in the circuit, as they formed the largest sub-population of MS rhythmic neurons which have been hypothesised to be the coordinators of hippocampal theta oscillations (Gogolák et al., 1968; Petsche et al., 1962; Stumpf et al., 1962). Teevra neurons have a short burst duration, do not change their firing rate from rest to run and fire action potentials at the trough relative to dorsal CA1 theta oscillations recorded in stratum pyramidale, coincident with the maximal firing of CA1 pyramidal cells (O’Keefe and Recce, 1993). The latter fact points to a role of Teevra cells in promoting increased hippocampal activity during theta oscillations.

3.8 Conclusion and next steps

I have identified distinct groups of MS neurons based on activity dynamics and selected the largest group, the Teevra cells, for testing the hypothesis that these neurons represent a distinct population in the septo-cortical circuit. To probe this, the activity patterns, molecular parameters, axonal termination areas, and synaptic target neurons of extracellularly recorded and juxtacellularly labelled Teevra cells were analysed, which will form a part of subsequent chapters.

Table 3.1

Cell id	Mean Rate (Hz)		Rate change score	Mean Burst Duration (ms)	Phase preference CA1 (deg)	Rhythmicity Index (R.I.)		Clustering (Ward Method)	
	RES T	RUN				RI	OF	Clu. Id	Sil. val
A42f	13.1	3.0	-0.6	40.9	242.0	0.3	6.1	1.0	0.9
M53y	35.2	14.5	-0.4	45.9	248.1	0.3	6.3	1.0	0.6
A19n	14.0	5.0	-0.5	36.2	194.9	0.1	n.a.	1.0	0.8
A45i	46.1	9.2	-0.7	25.3	214.9	0.1	n.a.	1.0	0.9
A43l	28.6	23.4	-0.1	39.4	52.3	0.1	7.7	2.0	0.9
A40g	35.5	25.5	-0.2	46.0	176.0	0.1	4.8	2.0	0.8
A52g	27.0	27.6	0.0	51.6	357.0	0.1	7.4	2.0	0.5
T68a	24.4	13.5	-0.3	45.7	68.4	0.2	4.9	2.0	0.2
A49d	27.0	26.6	0.0	49.2	172.9	0.2	7.4	2.0	0.7
A37i	42.3	43.3	0.0	37.1	33.3	0.2	7.6	2.0	0.8
A37x	21.0	15.8	-0.1	47.9	254.5	0.2	7.0	2.0	0.8
A40m	18.2	13.0	-0.2	41.2	255.9	0.2	7.0	2.0	0.8
A37z	46.6	40.1	-0.1	25.8	7.0	0.6	7.2	2.0	0.9
A48j	38.9	33.2	-0.1	26.3	83.6	0.3	6.8	2.0	0.9
A51b	36.2	23.4	-0.2	30.1	253.1	0.3	7.8	2.0	0.6
A40y	30.9	24.6	-0.1	32.4	121.3	0.3	6.9	2.0	0.9
M26g	37.1	35.2	0.0	36.7	310.9	0.3	6.8	2.0	0.9
M31h	20.5	20.6	0.0	39.1	115.0	0.3	6.2	2.0	0.8
A44k	39.1	24.1	-0.2	33.4	40.6	0.3	7.0	2.0	0.6
A43f	50.9	45.7	-0.1	35.4	284.7	0.4	5.9	2.0	0.9
A43n	67.1	63.1	0.0	46.9	320.5	0.4	7.4	2.0	0.8
A45h	28.5	33.4	0.1	49.1	5.7	0.5	6.8	2.0	0.1
A42k	28.7	20.5	-0.2	18.1	0.8	0.5	7.3	2.0	0.7
A43h	29.7	25.9	-0.1	40.7	261.7	0.6	7.2	2.0	0.9
A20f	61.0	60.9	0.0	31.7	38.4	0.6	7.8	2.0	0.8
A43e	78.3	66.0	-0.1	31.5	333.3	0.6	7.9	2.0	0.9
A43c	72.6	73.8	0.0	34.5	152.3	0.6	7.6	2.0	0.8
A37j	30.0	19.2	-0.2	41.5	282.9	0.2	7.7	2.0	0.7

A43a	45.5	55.2	0.1	30.2	59.8	0.6	7.6	2.0	0.5
A42m	72.4	61.6	-0.1	37.9	341.6	0.7	7.5	2.0	0.9
aj29h_3	77.6	72.1	0.0	24.4	210.0	0.1	7.0	2.0	0.8
aj27c_11	28.3	24.0	-0.1	45.4	306.6	0.1	7.0	2.0	0.9
aj29f_5	27.7	16.5	-0.3	51.0	2.5	0.1	7.0	2.0	0.5
aj23l_7	47.5	50.9	0.0	33.9	196.7	0.2	7.0	2.0	0.8
aj29g_2	15.2	11.7	-0.1	52.3	279.4	0.2	7.0	2.0	0.8
aj29f_7	29.2	22.4	-0.1	43.1	308.6	0.2	7.0	2.0	0.9
aj27c_10	30.5	27.4	-0.1	38.2	222.7	0.2	7.0	2.0	0.9
aj27c_14	48.4	40.1	-0.1	39.4	123.4	0.2	7.0	2.0	0.9
aj27c_6	102.7	108.9	0.0	21.5	69.8	0.2	7.0	2.0	0.7
aj31b_11	30.8	38.0	0.1	40.8	217.6	0.2	7.0	2.0	0.3
aj27b_7	30.4	19.6	-0.2	33.8	256.8	0.2	7.0	2.0	0.7
aj27c_13	56.5	52.0	0.0	37.2	6.0	0.3	7.0	2.0	0.9
aj29g_4	74.9	78.4	0.0	38.7	301.4	0.3	7.0	2.0	0.8
aj27b_13	8.8	8.5	0.0	39.0	301.9	0.5	7.0	2.0	0.8
aj27b_3	19.5	14.7	-0.1	27.5	36.9	0.5	7.0	2.0	0.8
aj27c_16	25.3	28.1	0.1	47.4	50.1	0.5	7.0	2.0	0.5
aj27b_12	66.9	49.9	-0.1	38.0	335.1	0.5	7.0	2.0	0.9
aj27c_7	66.9	66.0	0.0	38.0	63.4	0.6	7.0	2.0	0.8
aj27c_20	54.1	49.2	0.0	39.5	13.8	0.6	7.0	2.0	0.9
aj29f_4	22.8	29.2	0.1	37.8	352.5	0.6	7.0	2.0	0.3
aj27b_11	78.3	89.5	0.1	35.3	0.5	0.6	7.0	2.0	0.6
aj27c_17	34.2	31.9	0.0	40.0	342.2	0.7	7.0	2.0	0.9
M19b	33.0	43.7	0.1	52.4	170.4	0.1	5.5	3.0	0.6
A38p	25.0	62.7	0.4	66.7	214.3	0.1	6.9	3.0	0.7
A50h	25.3	32.9	0.1	63.4	268.6	0.1	7.6	3.0	0.7
A40l	22.8	33.7	0.2	69.4	238.9	0.1	7.3	3.0	0.8
A20l	41.9	63.6	0.2	60.6	207.0	0.2	7.9	3.0	0.9
M24a	34.9	49.5	0.2	47.8	180.4	0.2	7.1	3.0	0.6
A44j	41.0	66.4	0.2	67.2	133.5	0.2	8.0	3.0	0.8
A37o	20.7	32.9	0.2	56.2	225.6	0.2	7.1	3.0	0.9

A50j	45.6	61.1	0.1	55.3	188.5	0.2	7.7	3.0	0.7
A43j	35.3	63.5	0.3	57.1	160.9	0.3	8.0	3.0	0.9
aj27b_16	24.0	41.5	0.3	51.7	221.6	0.1	7.0	3.0	0.8
aj27c_22	24.4	32.2	0.1	66.7	121.1	0.1	7.0	3.0	0.7
aj27c_2	7.5	24.1	0.5	64.3	330.6	0.2	7.0	3.0	0.6
aj23l_10	13.8	30.1	0.4	55.0	100.5	0.2	7.0	3.0	0.9
aj27c_21	64.0	97.0	0.2	73.5	210.7	0.2	7.0	3.0	0.7
aj27b_4	12.2	16.4	0.1	60.0	359.6	0.3	7.0	3.0	0.7
aj27b_14	12.1	18.5	0.2	57.7	156.9	0.3	7.0	3.0	0.8
aj29h_9	14.7	23.5	0.2	53.0	119.8	0.3	7.0	3.0	0.8
aj27b_10	57.0	86.7	0.2	59.1	177.2	0.3	7.0	3.0	0.9
aj27b_5	8.7	20.5	0.4	61.7	340.1	0.4	7.0	3.0	0.8
aj29h_6	22.5	55.9	0.4	54.4	355.2	0.4	7.0	3.0	0.8
aj27c_15	25.9	35.2	0.2	47.5	122.4	0.5	7.0	3.0	0.4
aj27c_12	40.7	63.1	0.2	44.0	197.3	0.6	7.0	3.0	0.6
A42l	20.1	31.9	0.2	118.3	163.9	0.1	7.5	4.0	0.7
A50a	12.2	13.6	0.1	99.2	297.8	0.1	7.5	4.0	0.5
A50i	38.0	72.4	0.3	81.1	118.9	0.1	7.8	4.0	-0.4
T78k	34.0	65.8	0.3	110.2	139.0	0.1	4.9	4.0	0.7
M20e	19.2	37.1	0.3	87.8	62.4	0.2	5.8	4.0	0.1
T62c	25.3	40.3	0.2	82.6	246.6	0.2	6.6	4.0	-0.3
aj23l_3	6.2	11.9	0.3	138.8	91.4	0.2	7.0	4.0	0.7
aj29g_6	9.5	11.0	0.1	86.6	258.4	0.3	7.0	4.0	0.0
aj31c_8	9.1	14.2	0.2	91.9	308.6	0.3	7.0	4.0	0.4
aj27b_9	8.3	23.4	0.5	97.4	248.4	0.3	7.0	4.0	0.4
aj31c_10	7.5	14.7	0.3	74.1	288.6	0.4	7.0	4.0	-0.7
aj27b_17	5.9	8.5	0.2	103.0	111.1	0.1	7.0	4.0	0.6
aj29h_10	12.1	13.2	0.0	105.0	246.1	0.3	7.0	4.0	0.5
aj27b_8	4.9	14.2	0.5	150.0	352.3	0.6	7.0	4.0	0.6

Table 3.1: Physiological parameters of clustered medial septal rhythmic neurons

All calculated parameters for clustered medial septal neurons show the within group similarity ‘silhouette values’ corresponding to the four clusters (cluster id 1 to 4, colour code). Neurons recorded using tetrode (lowercase cell id) or glass electrodes (uppercase cell id) are represented in all clusters except cluster 1. Neuron id T68a, T78k, T62c were recorded by Dr. Tim Viney and neuron id M20e, M53y, M31h, M24a, M19b were recorded by Mr. Minas Salib and are included to demonstrate the reproducibility of the recordings and the applicability of my analysis. Rhythmicity index (RI), mean burst duration, oscillatory frequency of burst firing (OF) and mean firing phase preference with respect to dorsal CA1 theta oscillations were calculated during RUN periods.

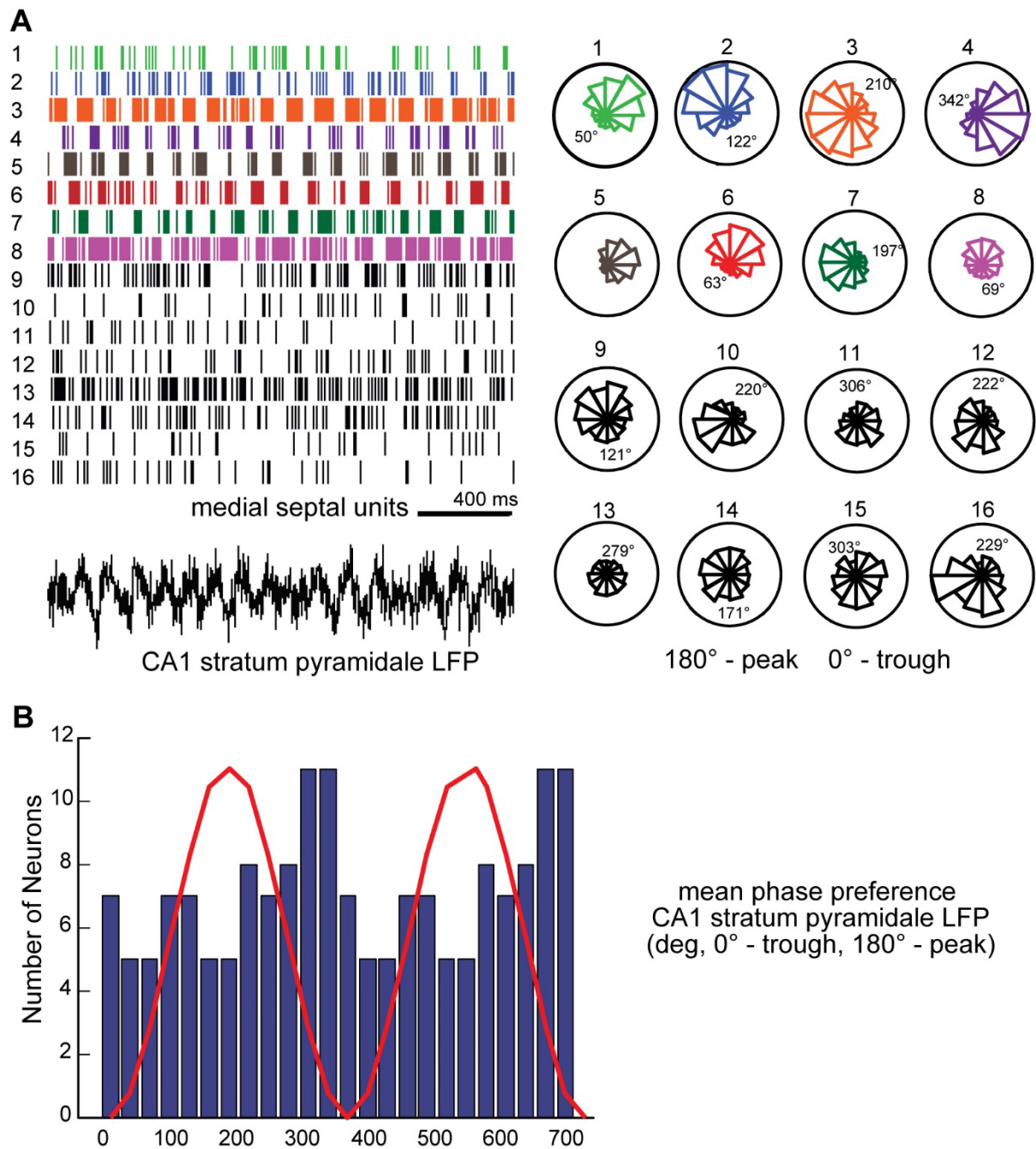


Figure 3.1: Medial septal neurons show diverse firing rates and phase preferences

(A) Left, Simultaneously recorded medial septal neurons ($n = 16$, recording id = aj27-0203c) exhibit high variation in firing rates (Mean $\pm$ SD = 45.1 ± 28.1 Hz, during RUN). **Right**, Firing phase distribution diagrams relative to hippocampal theta recorded in the pyramidal layer of CA1 (bottom left); the preferential firing phases of neurons cover the entire theta cycle. **(B)** Mean theta-related firing phase distribution of all medial septal neurons ($N = 4$ animals, $n = 81$ neurons, 30° bins) spanning the entire cycle (schematic theta cycle in red; data duplicated digitally to display two cycles) (0° trough - 180° peak of dorsal CA1 st. pyramidale LFP).

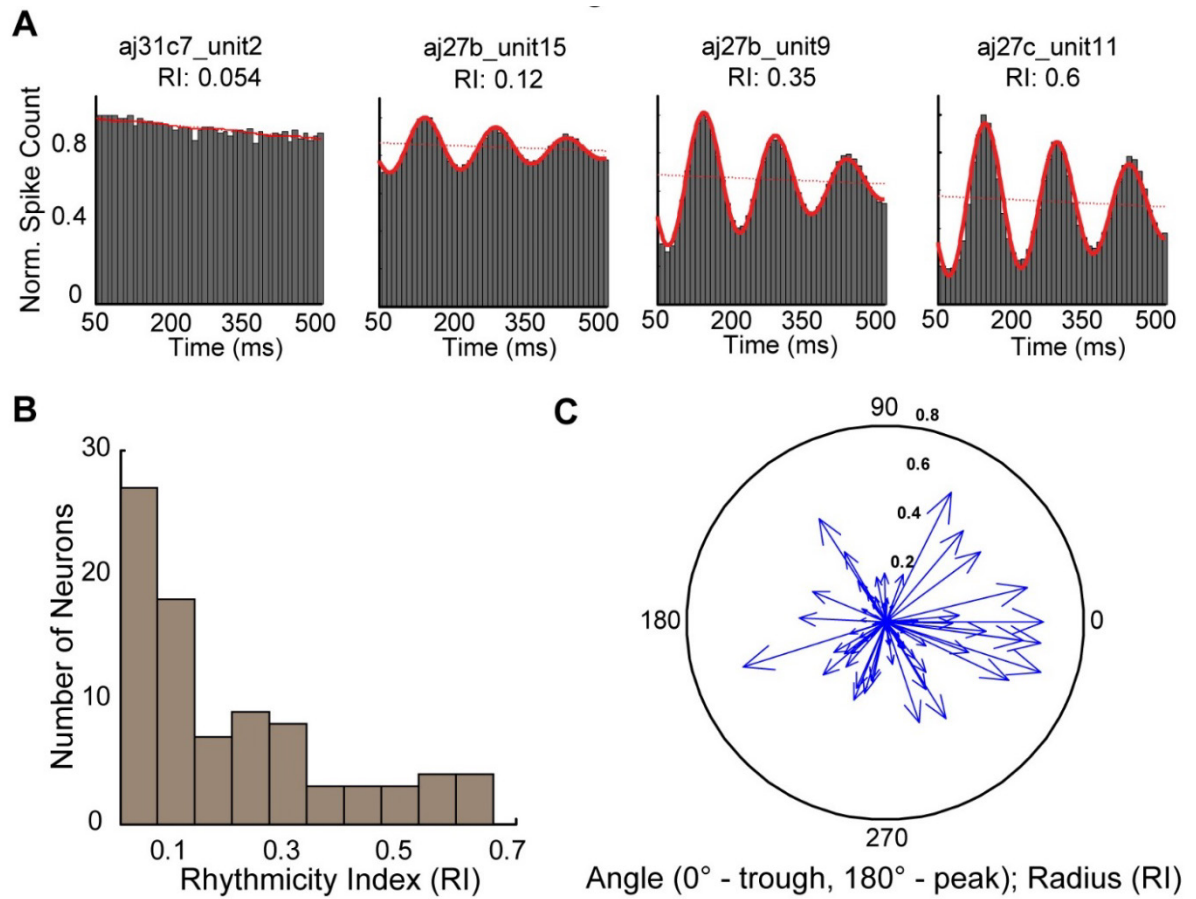


Figure 3.2: Rhythmicity index captures the regularity in the autocorrelation histogram

(A) Autocorrelation histograms of four neurons with varying levels of rhythmicity. Dotted line is a linear trend line and the fitting function is a Gaussian-modulated cosine function with three parameters (see Chapter 2). Calculated rhythmicity index (above histograms) reliably captures the rhythmic structure and depth of modulation of firing. (B) Distribution of the rhythmicity index of all recorded medial septal neurons ($n = 81$ neurons, $N = 4$ mice). (C) Polar plot depicting mean phase preference for each neuron with rhythmicity index as its radius. Note, neurons with high rhythmicity may be represented at many phases of the theta cycle, but were more frequently represented near the trough (0° trough - 180° peak of dorsal CA1 st. pyramidal LFP).

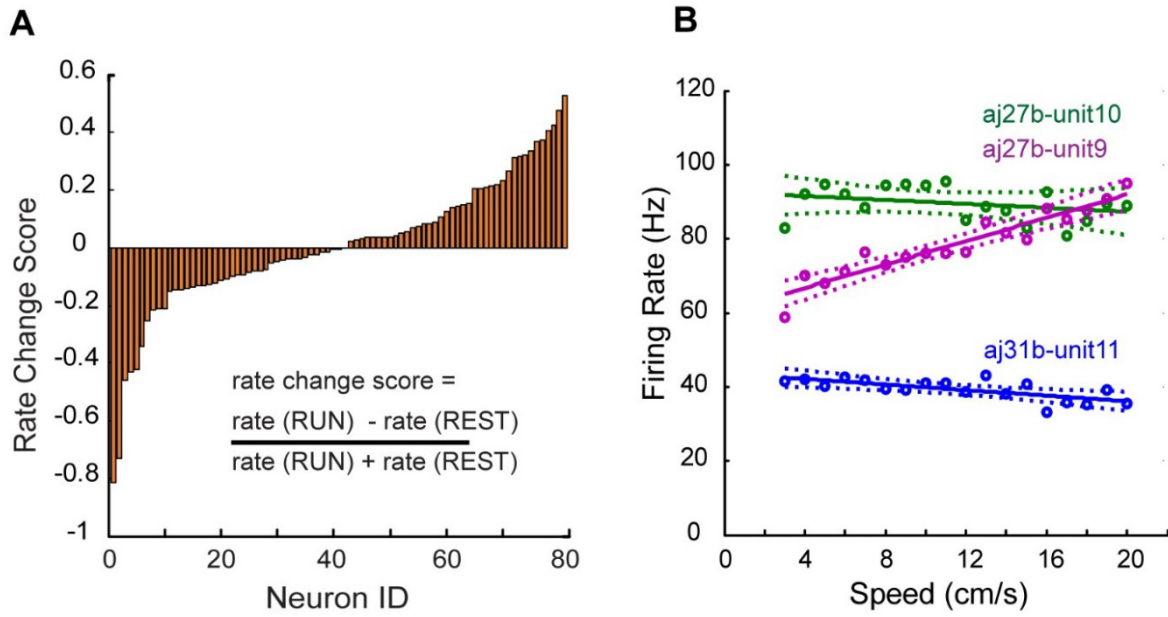


Figure 3.3: The firing of medial septal neurons is modulated by running speed

(A) Rate change score (ranges from -1 to 1) for all recorded neurons is widely distributed (mean $\pm$ SD = 0.006 ± 0.25). Each vertical bar represents the score for an individual neuron. **(B)** Correlation of firing rate with running speed of the animal for three recorded neurons: aj27b-unit9 increases its firing rate with the running speed (purple, $p < 0.005$, weighted fitting function $f = 60.5 \text{ Hz} + 1.6 \times s$; where s is the speed), aj31b-unit11 decreases its firing rate with running (blue, $p < 0.005$, weighted fitting function $f = 43.6 \text{ Hz} - 0.37 \times s$; where s is the speed) and aj27b-unit10 is not modulated by running speed (green, weighted fitting function $f = 92.6 \text{ Hz} - 0.26 \times s$; where s is the speed).

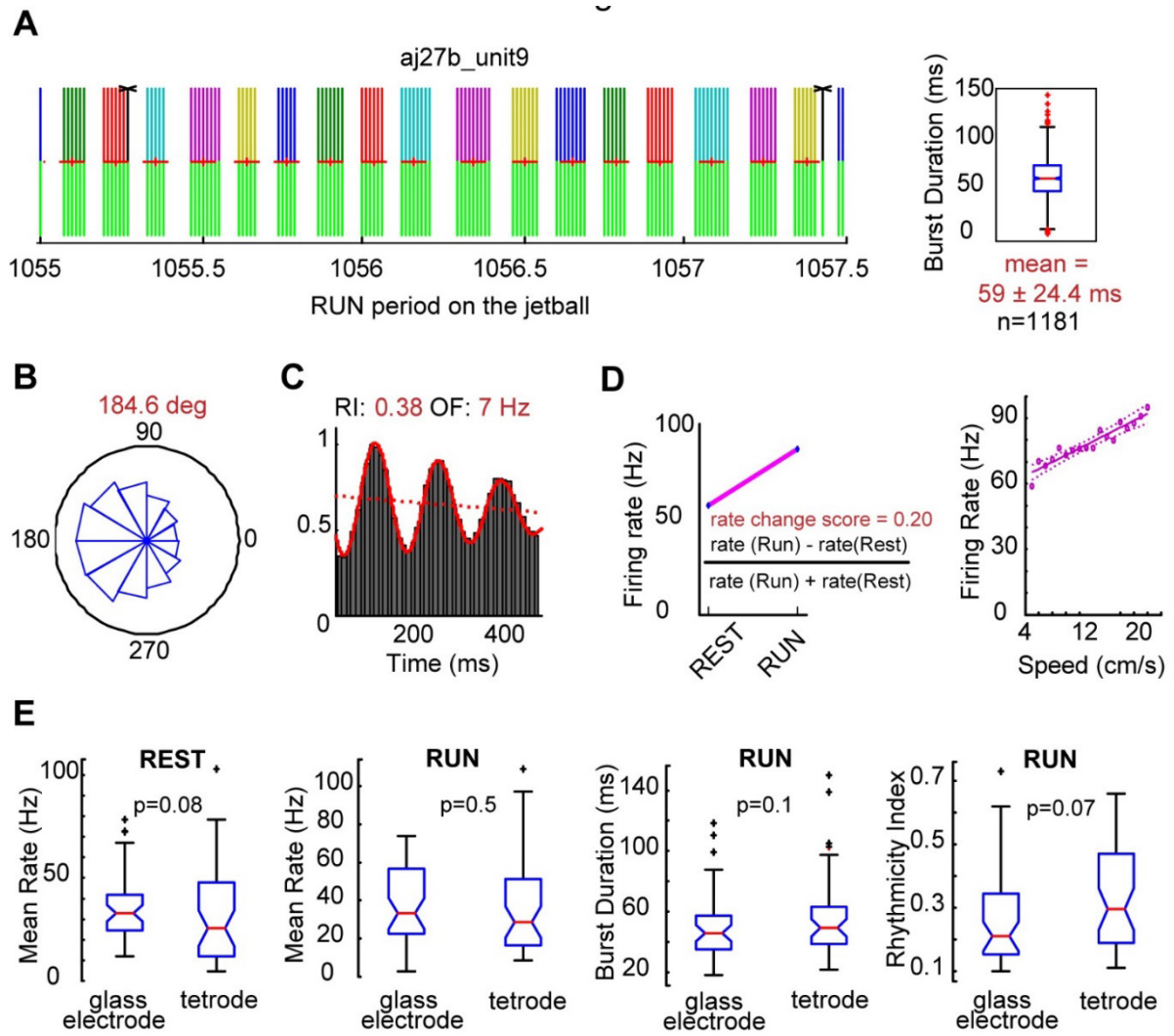


Figure 3.4: Firing parameter calculations based on spike-train dynamics

(A) **Left**, Spikes (vertical lines) are grouped as a ‘burst’ by the algorithm (common colour code). Note, some spikes are rejected as not being part of a ‘burst’ (black cross above spike). **Right**, Distribution of burst durations during RUN period on the jetball. (B) Circular plot of firing phase distribution with mean phase preference (top) of the neuron with respect to dorsal CA1 st. pyramidal theta oscillation. (C) Rhythmicity index (RI) calculated based on the autocorrelation histogram (50-500ms, 10ms bins) during RUN periods. (D) **Left**, Neuron (aj27b_unit9) increases its firing rate during RUN compared to REST resulting in a rate change score of 0.20. **Right**, Within the RUN epoch, the same neuron increases its firing rate with the running speed of the animal which can be depicted by a weighted fitting function $f = 60.5 \text{ Hz} + 1.6 \times s$; where s is the speed, $p < 0.005$. (E) Comparison of parameters calculated based on the spike train dynamics of neurons recorded using glass electrode or silicon probe recordings ($p > 0.05$, Kruskal-Wallis test). +, outliers.

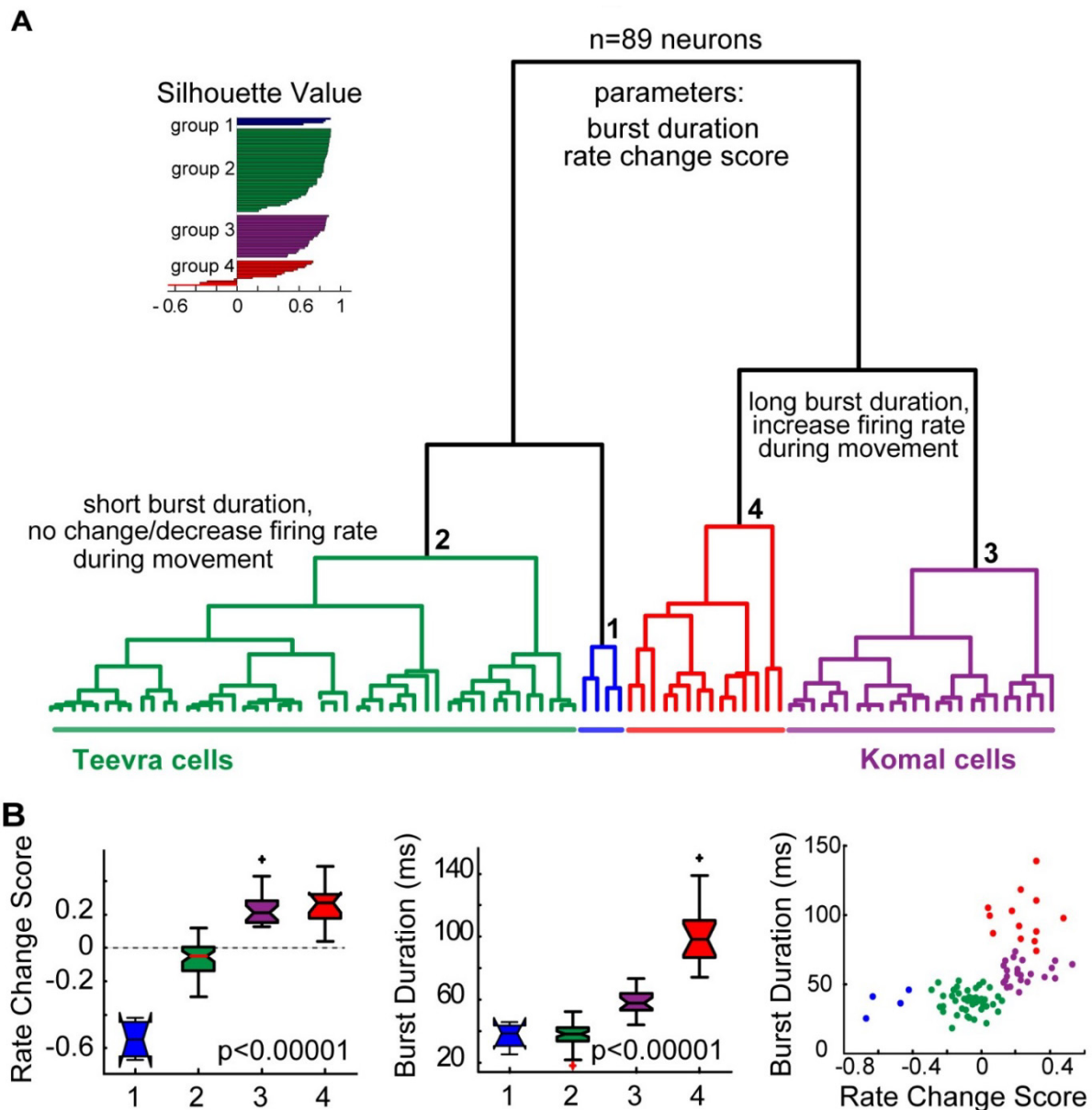


Figure 3.5: Subpopulations of medial septal rhythmic neurons based on spike train dynamics

(A) Hierarchical clustering of strongly rhythmic medial septal neurons ($RI > 0.1$, $n=89$) into four groups based on rate change score and burst duration as parameters. Left, Silhouette values show high intra-cluster similarity. The silhouette value (range, -1 to 1) indicating group cohesion for each point is a measure of how similar that point is to points in its own cluster vs. points in other clusters. (B) *Left*, Comparison of rate change score and burst duration for the four groups (median values, Kruskal-Wallis test). *Right*, Distribution of individual neurons clustered according to their colour code (Teevra cells, green; Komal cells, magenta).

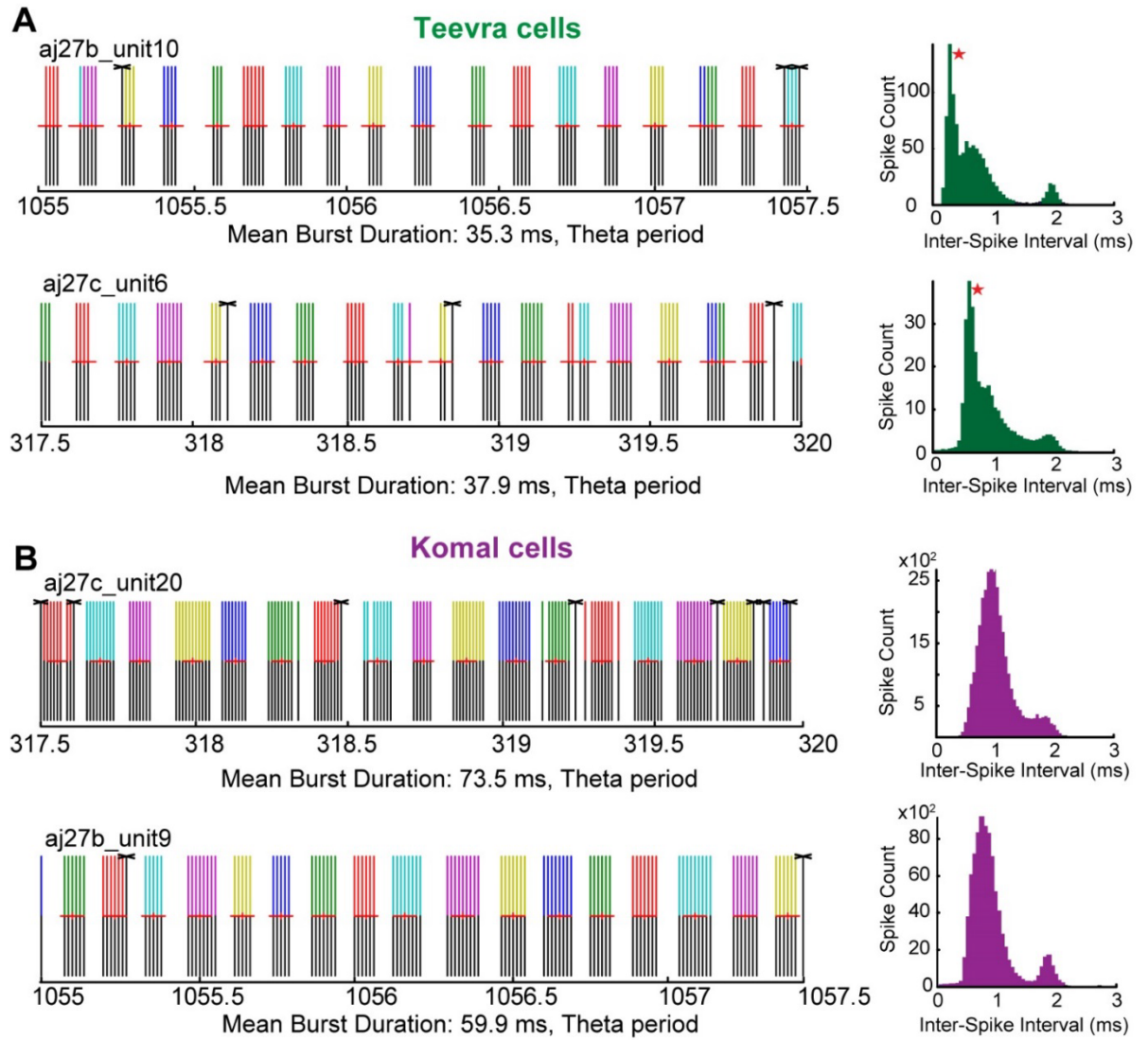


Figure 3.6: Teevra and Komal cells – burst duration

(A, B) simultaneously recorded Teevra and Komal cells show differences in their burst duration during theta oscillations. Top, detected bursts are shorter for Teevra cells. Right, inter-spike interval histogram of Teevra cell displays an additional peak at <5ms (asterisk) compared to Komal cell. Note, aj27b_unit10 and aj27b_unit9 are simultaneously recorded. Thus, Teevra cells could be distinguished from Komal cells based on their short mean burst duration during RUN periods.

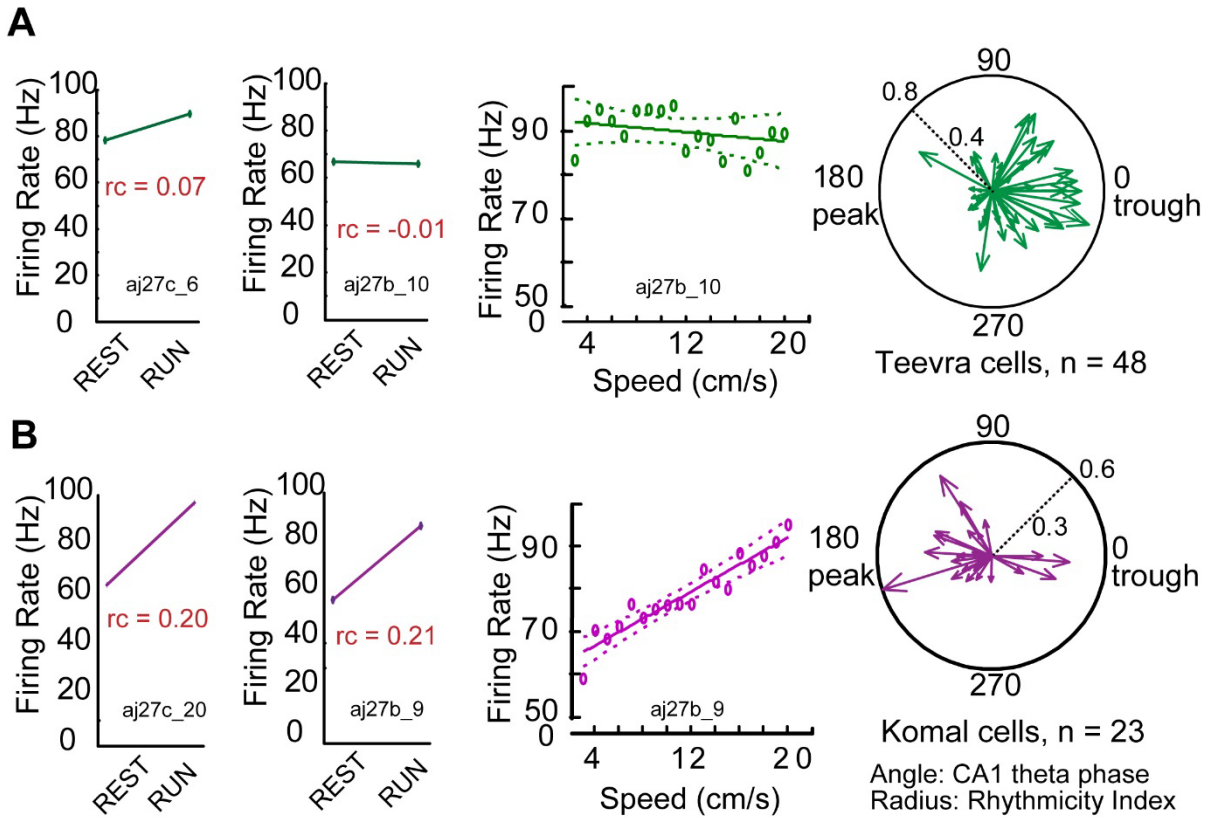


Figure 3.7: Behavioural- and network- state dependence of Teevra and Komal cells

Left, Teevra cells (**A**) do not change their mean firing rate from REST to RUN whereas Komal cells (**B**) have a characteristic increase in mean firing rate during RUN compared to REST. **Middle**, During the RUN period the firing rate of a Teevra cell (**A**) is not modulated by running speed (green, weighted fitting function $f = 92.6 \text{ Hz} - 0.26 \times s$; where s is the speed), whereas that of a Komal cell (**B**) increases with running speed (purple, $p < 0.005$, weighted fitting function $f = 60.5 \text{ Hz} + 1.6 \times s$; where s is the speed, dashed lines, 95% confidence interval). **Right**, Preferential theta phase of firing of Teevra and Komal cells with rhythmicity index as the radius (RUN periods). Most Komal cells (purple) fire preferentially at the peak of CA1 pyramidal cell layer theta oscillations, whereas most Teevra cells (green) fire phase coupled to the trough with increasing rhythmicity index.

Chapter 4: Physiological and molecular features of Teevra cells

4.1 Introduction

Hierarchical cluster analysis of MS neurons revealed the most populous cluster of septal cells, the Teevra cells, which rhythmically fire short bursts of action potentials coupled to the trough of hippocampal theta oscillations (Chapter 3). An intriguing feature of Teevra cell firing was that they exhibit negligible change in their mean firing rate as the behavioural state changed from REST to RUN. Data on medial septal neuronal activity in awake animals is sparse (Dragoi et al., 1999; Justus et al., 2017; King et al., 1998; Tsanov et al., 2014). King et al. showed one example of a MS neuron of unknown neurotransmitter phenotype, which increased its burst frequency during running (King et al., 1998). Justus et al. showed a few examples of medial septal neurons that increased, decreased or did not change their firing rates (the latter similar to Teevra cells) with increasing running speed of the animal, and suggested that the MS *glutamatergic* neurons send ‘speed’ signals to the medial entorhinal cortex (Justus et al., 2017). It has recently been shown that the medial entorhinal cortex shows two kinds of speed modulations or ‘signals’, one of which is of medial septal origin from unidentified cell type/s (Hinman et al., 2016). During my thesis work, I have also observed speed modulated medial septal cells (e.g. Komal cells, Chapter 3). If speed modulated medial septal neurons are involved in broadcasting running related information to cortical areas, what ‘information’ do Teevra cells provide? In this chapter, I will explore additional physiological parameters of Teevra cells during well-defined behavioural states to understand their contribution to the hippocampal chronocircuit (Somogyi, 2010). The rhythmicity index (RI) and oscillatory frequency of burst firing (OF) provide a quantifiable measure to assess the change in spike-train patterns from REST to RUN. Additionally, during REST, the hippocampal LFP is known to be dominated by

large amplitude irregular activity (LIA) (O’Keefe, *The hippocampus Book*, p475) that appears to be non-rhythmic and is in contrast to the highly regular theta oscillations during running (Jarosiewicz and McNaughton, 2002). I have tested if medial septal Teevra cells correlate their activity with the LIA.

Are Teevra cells a distinct cell type according to the definition provided in Chapter 3? They clustered according to similarity in activity patterns (burst duration during RUN and rate change score) and their temporal influence in the hippocampal chronocircuit seems to be mainly at the trough of dorsal CA1 pyramidal LFP during RUN (Fig. 3.7). Here, in addition to exploring parameters based on their spike-train dynamics, I will assess whether labelled Teevra cells can be characterised by a distinct molecular profile and if they deliver the same neuroactive substances to their synaptic targets. A majority of identified medial septal rhythmic neurons have been found to be PV-immunopositive (Borhegyi et al., 2004; Hangya et al., 2009; Simon et al., 2006). However, distinct subpopulations might exist within the medial septal PV⁺ population as it is the case in other circuits including the hippocampus (Klausberger and Somogyi, 2008). Hence, I have assessed three additional proteins previously used to segregate some hippocampal interneuron subpopulations (Somogyi et al., 2014; Viney et al., 2013), namely the transcription factor special AT-rich sequence-binding protein-1 (SATB1), the metabotropic glutamatergic receptor type-1 (mGluR1a), and the neurokinin-1 receptor (NK1R). Neurotransmitters are stored in synaptic vesicles before exocytotic release to the synaptic cleft. Do labelled Teevra cells express vesicular transporter(s) that can identify their transmitter phenotype? Here I assessed this using immunoreactions for vesicular GABA transporter (VGAT) and vesicular glutamate transporter (VGluT2).

4.2 Experimental setup

Single cell extracellular recording was performed in head-fixed mice running on a spherical treadmill. Out of a total of 31 mice that I trained and recorded from, 11 experiments resulted in successful labelling of the targeted and recorded neuron. In other experiments, either a labelled cell could not be found or it was not possible to attribute the recorded physiological activity to the labelled cells due to multiple labelling. Teevra cells could be labelled in 8 successful experiments. Dorsal CA1 str. pyramidale neuronal activity was recorded simultaneously in all experiments and served as a temporal reference across experiments. Once the recorded neuron was identified as a Teevra cell based on the ‘sound’ of their burst firing (Video 4.1) and the relationship with running speed of the animal, they were juxtacellularly labelled by injecting current pulses in the glass electrode just next to the cell (‘juxtacellular’) using 3% neurobiotin in 0.5 M NaCl in the recording electrode. Neurobiotin is then visualised by adding a fluorescence probe conjugated to the protein avidin in 70 – 80 μm thick brain sections (Fig. 4.1A).

Having identified distinct groups of MS neurons based on activity dynamics, the largest group, the Teevra cells, which had the highest rhythmicity index (median: 0.3, IQR: 0.18 – 0.55, $n = 48$) was selected for testing the hypothesis that these neurons represent a distinct population in the septo-cortical circuit. In this chapter, I present data from 13 labelled Teevra cells (Table 4.1; 5 cells labelled by colleagues and analysed by me), enabling the assessment of molecular parameters of the recorded cells.

4.3 Rhythmicity index and oscillatory frequency of Teevra cells increases during RUN

The rhythmicity index (RI) was computed during running and resting. Teevra cells discharged rhythmic bursts during RUN and REST periods, but the extent of rhythmicity increased during running (median RI run: 0.3, IQR: 0.18 – 0.55; median RI rest: 0.07, IQR: 0.04 – 0.1, Wilcoxon

paired-sample test, $p = 1.6 \times 10^{-09}$). Periods of RUN are dominated by theta oscillations (7 – 9 Hz under these conditions) and REST periods are dominated by irregular activity. Consistent with the increase in theta frequency during running (Sławińska and Kasicki, 1998), the oscillatory frequency (OF) of Teevra cells also increases during running (median OF run: 7 Hz, IQR: 7 – 7.34 Hz, median OF rest: 6.4 Hz, IQR: 6.04 – 6.5 Hz, Wilcoxon paired-sample test, $p = 4.9 \times 10^{-7}$). Examples of juxtacellularly labelled Teevra neurons, AJ42m and AJ45h (Fig. 4.1B and Fig. 4.1C, Table 4.1) show such an increase in rhythmicity index and in the oscillatory frequency of burst firing. Note, the peak in firing in the 50 – 200 ms range in the autocorrelation histograms shifts to the lower values during RUN (Fig. 4.1), demonstrating a higher oscillatory frequency.

4.4 Increased activity of Teevra cells is related to hippocampal LFP during REST

I next explored the activity patterns of Teevra cells during REST periods when large amplitude irregular activity (LIA) dominates CA1 field potential oscillations. The LFP crossings at 0 mV (zero crossings) of the LIA were detected from the down-sampled (1 kHz) CA1 reference LFP channel at the falling phase (minimum interval between consecutive zero crossings: 200 ms). Interestingly, Teevra cells couple to the falling transition, as measured at zero crossing of the LIA during REST periods (Fig. 4.2A) mirroring the trough coupling during RUN and following the duration of the slow cycle. Thus, Teevra cells become active at hippocampal troughs, coincident with the heightened excitation in CA1 (Mizuseki et al., 2009), irrespective of the frequency at which they occur (during theta or during slow 2 to 6 Hz irregular activity). Teevra cells maintained a rhythmic autocorrelation histogram during REST periods. They resulted in an oscillatory frequency of approximately 5 to 6 Hz, which explains the band of action potentials between two consecutive zero crossings (Fig. 4.2A).

Medial septal GABAergic cells could be active or inhibited during 130 – 240 Hz sharp wave ripple (SWR) oscillations which last for 50 – 200 ms (Borhegyi et al., 2004; Dragoi et al., 1999; Viney et al., 2013). Under the conditions of my recordings, SWRs were infrequent but could be observed for a few Teevra cells ($n = 4$), which did not change their firing significantly during ripple events (Fig. 4.2B) ($p > 0.05$, two-sample KS test, (Katona et al., 2014)).

4.5 Teevra cells are GABAergic, immunopositive for parvalbumin and SATB1 but not for mGluR1a

Teevra cells comprised a distinct subpopulation of MS neurons based on physiological parameters. Next I have tested whether they represent a distinct subpopulation according to molecular markers and transmitter phenotype. Labelled Teevra cells were immunopositive for the calcium binding protein parvalbumin (PV, $n = 9/10$ tested, Fig. 4.3A and Fig. 4.3C, Table 4.1), the transcription factor SATB1 ($n = 10/10$ tested, Fig. 4.3B and Fig. 4.3C), but lacked detectable immunoreactivity for mGluR1a in the somatic plasma membrane ($n = 10/11$ tested, Fig. 4.3B and Fig. 4.3D). A weak cytoplasmic signal may represent a pool of receptor in the endoplasmic reticulum. Approximately half of the PV⁺ neurons were immunopositive for SATB1 in the entire medial septal complex. I observed all four possible combinations of immunoreactivity for PV and mGluR1a, including double-immunonegative neurons. Teevra cells ($n = 5/5$ tested) were immunonegative for the NK1 receptor in their somato-dendritic membrane. Labelled Teevra cells were also tested for molecular phenotype of their boutons; all tested cells ($n = 4/4$, Table 4.1) were immunopositive for VGAT but not VGluT2 (Fig. 4.3E) confirming that they were GABAergic and not glutamatergic neurons.

Teevra cells emitted axonal collaterals and made boutons in the MS ($n = 5/5$ tested). These collaterals targeted mainly PV⁺ soma or dendrites ($n = 10/11$ tested targets, AJ42m axon, Fig. 4.3F, Table 5.1). All of the tested neurons innervated on their soma in the MS were PV⁺ and SATB1⁺ ($n = 5/5$). Varicosities of the axon of Teevra cell AJ42m were tested for the presence

of synapses using labelling for the postsynaptic junction scaffolding protein gephyrin (Lardi-Studler et al., 2007) and the majority of boutons formed GABAergic synapses ($n = 42/47$ tested varicosities, Fig. 4.3F).

4.6 Discussion

Teevra cell firing is temporally modulated over multiple timescales

A fascinating feature of the firing of Teevra cells is that the firing rate did not change from rest to running significantly or even decreased during the RUN periods for some cases. This is in contrast to the firing of most CA1 interneurons, which increased their firing rate during running periods as compared to when the animal was at rest (Czurkó et al., 2011; Varga et al., 2012). This was possibly due to a decreased inhibition from MS GABAergic cells, an increased excitatory input from CA3 pyramidal cells, and/or other long-range inputs (Fuhrmann et al., 2015). Although the firing rate of Teevra cells may not be different during REST and RUN periods, the temporal dynamics of firing pattern changed with a clear increase in the rhythmicity index and oscillatory frequency during running. If on average each action potential provides a similar amount of GABA released at the synaptic terminals, why do Teevra cells maintain a high level of GABA release during REST when their targets are much less theta rhythmic? In this chapter, I showed that Teevra cells continue to fire at the negative deflections of slow irregular activity, increasing their firing around the hippocampal LFP troughs, irrespective of the frequency at which these occur, mirroring the heightened excitation in CA1.

Sharp wave ripple (SWR) episodes are more frequent during rest and consummatory behaviour representing the highest population activity in the hippocampus. During SWRs some MS GABAergic neurons are strongly active (Borhegyi et al.; Viney et al., 2013) while others are inhibited (Borhegyi et al., 2004; Dragoi et al., 1999) or do not change their firing like Teevra cells. Some GABAergic neurons in the MS are innervated by hippocampo-septal GABAergic

projection neurons (Tóth et al., 1993), which are strongly activated during SWR events (Jinno et al., 2007; Katona et al., 2017). These MS cells might correspond to the SWR inhibited rhythmic population (Dragoi et al., 1999). Teevra cells on the other hand did not change their firing rate during SWRs suggesting that these may not be targets of hippocampo-septal projection neurons. This analysis shows that during REST periods, Teevra cells differentiated between the short duration SWR periods (no increase in firing) and the less regular longer duration increased hippocampal excitability events, when their firing was strongly coupled to hippocampal LFP troughs during LIA.

Molecular characteristics of Teevra cells

Teevra cells are GABAergic since they express the transporter of the amino acid, GABA and are immunonegative for VGluT2, which is expressed by glutamatergic medial septal neurons (Sotty et al., 2003). In addition, Teevra cells express PV, SATB1 and have undetectable levels of mGluR1a in their somatic and proximal dendritic membrane. This molecular combination in the septum could be a triple marker combination to identify Teevra cells. I am not aware of known molecular combinations that could identify distinct subpopulations of medial septal neurons. I hypothesise that other PV⁺ medial septal cells that exhibit activity patterns different from Teevra cells (like Komal cells) exhibit a distinct molecular marker combination. These might reflect their transcriptional architecture and help future studies aimed specifically at determining the transcriptome of various medial septal cell types along the lines of similar studies in the mouse cortex (Zeisel et al., 2015). The identity of the PV⁺ MS neurons innervated by Teevra cells remains to be determined. They may be other Teevra cells synchronized by local interconnections (Leao et al., 2015), GABAergic MS neurons projecting to other cortical areas, or different types of interneuron as suggested by Borhegyi et al. (2004).

4.7 Conclusion

Individual Teevra cells in the group deliver the same neuroactive substance (GABA) in the same temporal patterns (troughs of theta and LIA) in a brain state-specific manner (do not change their firing rate from REST to RUN significantly). This suggests that they are a distinct cell type. However the range of their influence, determined by their target-area and synaptic partners is not yet presented. The challenge is to obtain strong labelling of their projection axons to branches and terminals in order to identify their target area and synaptic partners. In chapter 5, I will attempt to address these challenges.

Teevra Cell Id	Rate (Hz)		Rate Change Score	Mean Burst Duration (ms)	Phase Preference (deg), dorsal CA1	Rhythmicity		Immunoreactivity			
	Rest	Run				RI	OF (Hz)	PV	SATB1	mGluR1a	VGAT
AJ42m ^R	72.4	61.6	-0.1	37.9	341.6	0.73	7.5	a+	n+	s-	t+
AJ37z	46.6	40.1	-0.1	25.8	7.0	0.61	7.2	u	u	u	u
AJ45h ^L	28.5	33.4	0.1	49.1	5.7	0.48	6.8	s+	n+	s-	t+
AJ43n	67.1	63.1	0.0	46.9	320.5	0.43	7.4	d+	n+	s-	t+
MS31h	20.5	20.6	0.0	39.1	115.0	0.30	6.2	u	u	u	u
AJ40y ^R	30.9	24.6	-0.1	32.4	121.3	0.28	6.9	d+	n+	s-	u
AJ48j ^R	38.9	33.2	-0.1	26.3	83.6	0.26	6.8	s+	n+	d-	t+
AJ52g ^R	27.0	27.6	0.0	51.6	357.0	0.14	7.4	s+	n+	s-	u
AJ43l ^R	28.6	23.4	-0.1	39.4	52.3	0.10	7.7	s, a-	n+	s+	u
MS11b*	31.8	39.7	0.1	56.0	320.3	0.01	6.1	u	u	d-	u
MS71c ^L	47.6	49.4	0.0	42.3	34.1	0.14	7.1	s+	n+	s-	u
MS73c ^L	52.5	34.6	-0.2	36.0	75.0	0.23	6.3	s+	n+	s-	u
MS90g*	36.3	32.7	0.05	44.5	138.6	0.29	7.9	a+	n+	s-	u

Table 4.1. Physiological parameters and immunohistochemical analyses of identified Teevra neurons

Positive (+) or negative/undetectable (-) immunoreactivity observed within subcellular domain: s, soma; n, nucleus; d, dendrite; a, axon; t, axon terminals; u, unavailable or inconclusive. Not shown: AJ42m, AJ43l, AJ45h, AJ48j, AJ52g are immunonegative for NK1 receptor (s,d) and AJ42m, AJ43l, AJ48j, AJ45h are immunonegative for VGluT2 (t). *Animal was not trained. These cells were not included in clustering due to absence of voluntary movement. Superscripts denote location of soma and axonal terminal in the right (^R) or left (^L) hemisphere. RI, rhythmicity index; OF, oscillatory frequency, RUN periods. MS11b, MS31h, MS71c, MS73c, MS90g are Teevra cells recorded by Mr. Minas Salib and included here to demonstrate the reproducibility of the physiological parameters calculated and molecular parameters observed.

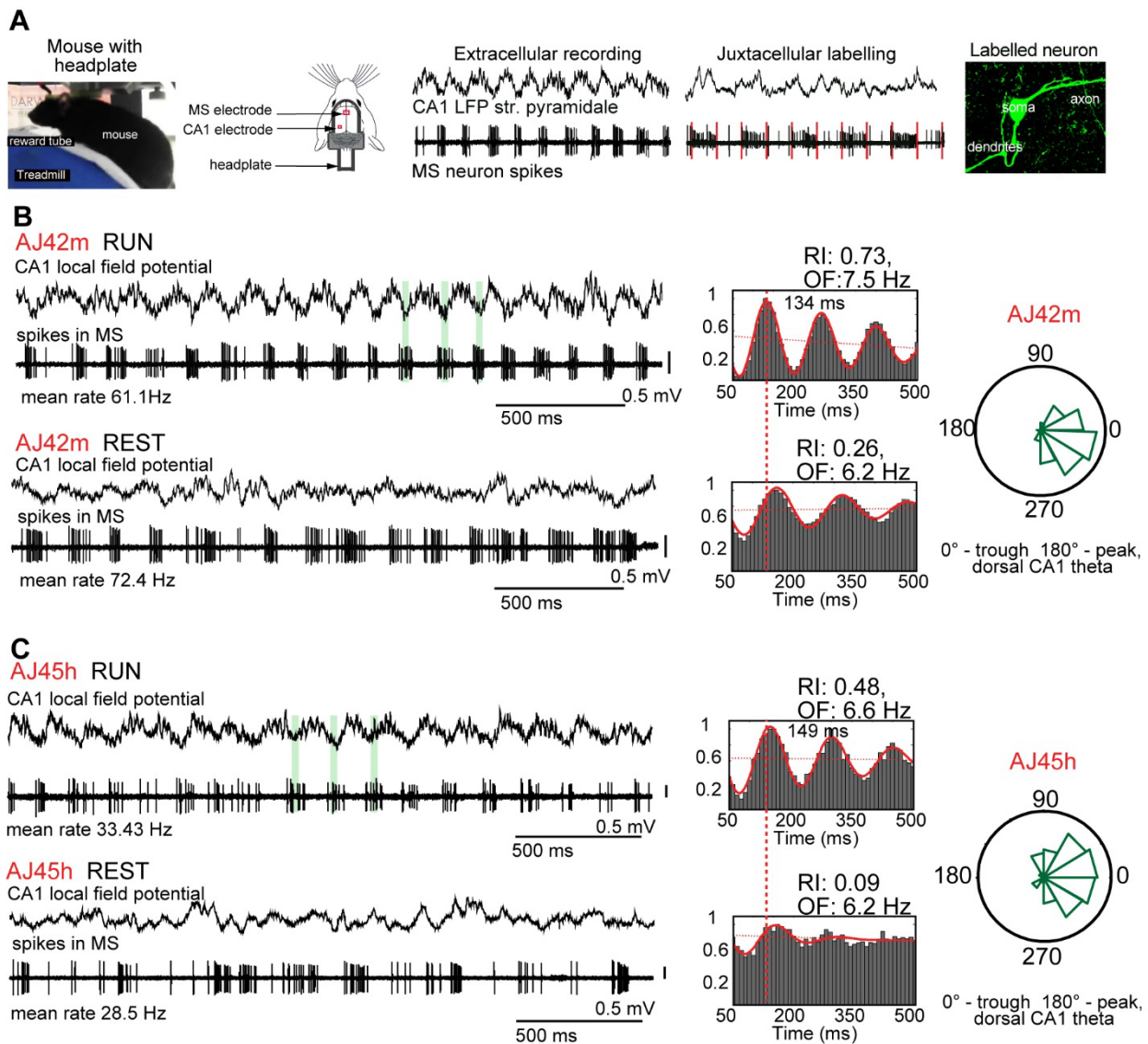


Figure 4.1: Teevra neurons increase their rhythmicity and oscillatory frequency of firing during RUN

(A) Single MS neurons and CA1 LFP were recorded extracellularly using glass electrodes in mice alternating between REST and RUN periods on a spherical treadmill. Teevra neurons preselected based on the short burst duration were juxtacellularly labelled with neurobiotin (green). (B, C) **Left**, Rhythmic burst firing of Teevra cells (AJ42m and AJ45h) during RUN (top) and REST (bottom). Local field potential (LFP) in CA1 stratum pyramidale. Some of theta trough-centred bursts of MS neuron action potentials relative to CA1 theta oscillations are highlighted (light green). **Middle**, Autocorrelograms show a slight increase in oscillatory firing frequency (OF) and large increase in the rhythmicity index (RI) during RUN (top) compared to REST (bottom). **Right**, Preferential coupling of spikes to CA1 theta troughs during RUN.

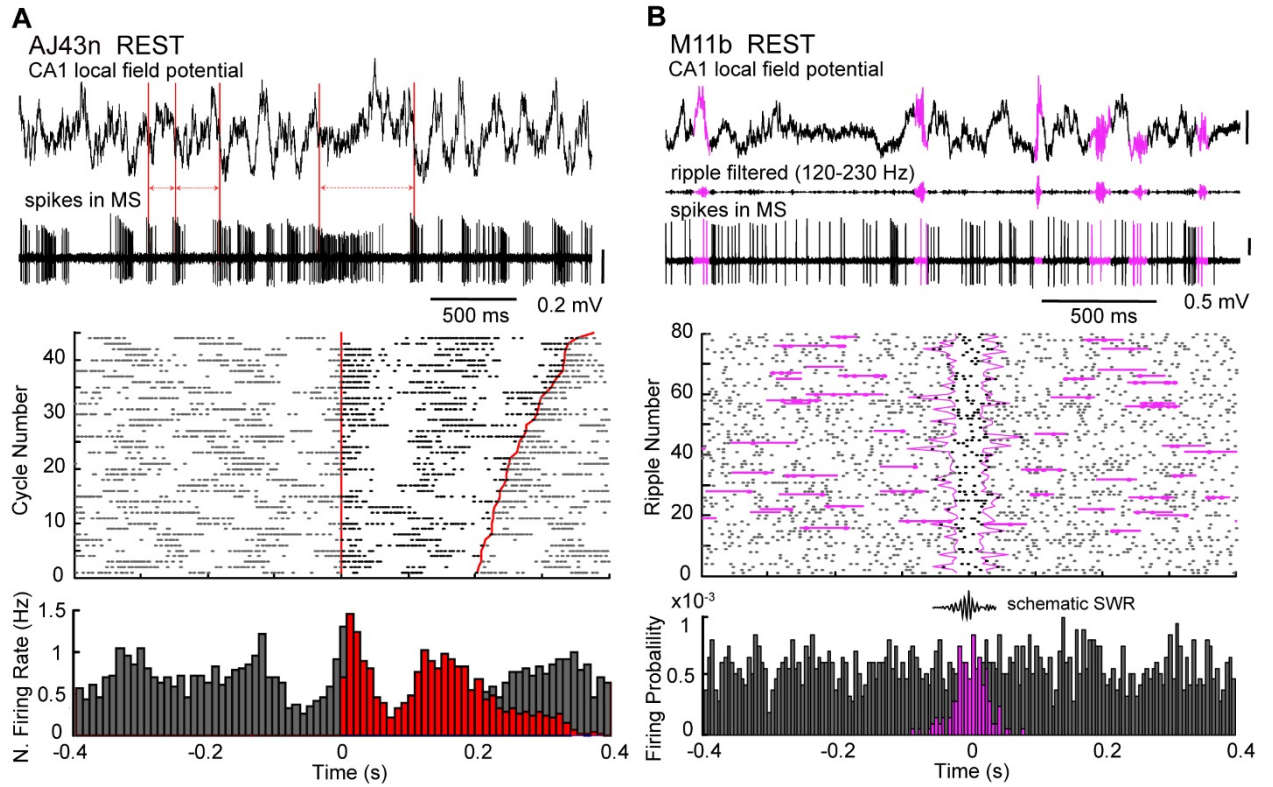


Figure 4.2: Teevra neurons fire coincident with increased hippocampal CA1 excitation

(A) Rhythmic burst firing of a Teevra neuron (AJ43n) during REST, a period dominated by large amplitude irregular activity (LIA) in CA1 stratum pyramidale (top). Two consecutive zero crossings at falling transition of the LFP (red lines) are marked. Spike raster plot (middle) and normalized spike firing histogram (bottom) show correlation of spikes (grey dots) with the timing of slow LFP oscillation cycles. Consecutive LFP zero-crossings (> 200 ms apart) are ordered according to their duration and marked by red lines; time 0 is the zero crossing of LFP falling transitions. Note additional burst at ~ 6 Hz between time points marked by red lines. (B) During ripple oscillations (pink, top), a Teevra neuron (MS11b) does not change its firing probability. Spike raster plot (middle) and firing probability (bottom) show sustained activity during ripple epochs (pink bars in the histogram) compared to ± 0.4 s (grey) surrounding the peak of SWR events. Raster plots were aligned to the peak SWR power and pink lines delineate the beginnings and end of SWRs. Time 0 is the peak power of each ripple oscillatory event.

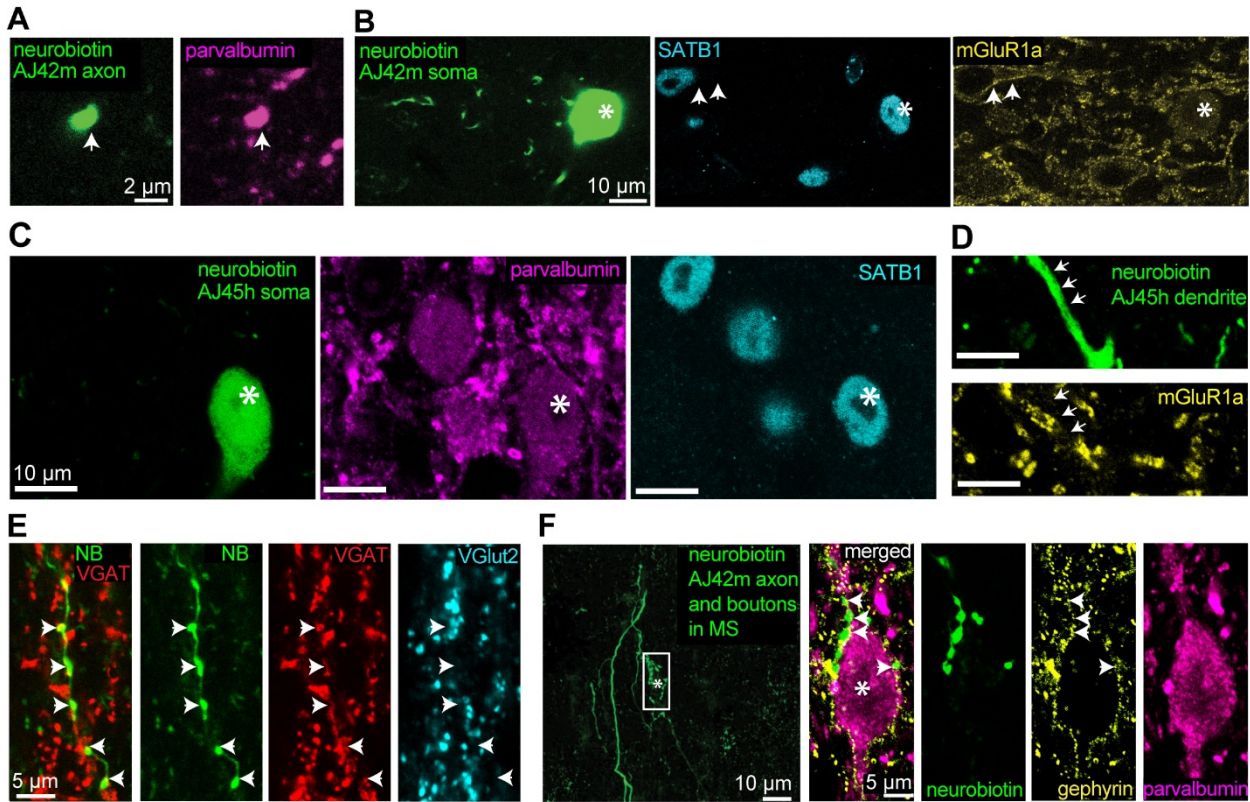


Figure 4.3: Teevra neurons are GABAergic, innervate PV⁺ neurons in the septum

(A, B) Neurobiotin labelled Teevra neuron, AJ42m was PV⁺ (magenta, main axon), SATB1⁺ (cyan, asterisk, nucleus), and immunonegative for mGluR1a in the plasma membrane (yellow) showing only a weak cytoplasmic signal. Note, mGluR1a⁺ somatic membrane labelling (yellow, arrows) of a neighbouring SATB1⁺ cell. (C, D) Neurobiotin labelled Teevra neuron, AJ45h is immunopositive for PV, SATB1, and immunonegative for mGluR1a in the dendritic membrane, similar to AJ42m. (E) Axon terminals of AJ42m (green) were VGAT⁺ (red, arrows) and VGlut2⁻ (cyan, arrows). (F) Main axon and local terminals of AJ42m in the MS (green, box) innervating a PV⁺ soma (magenta, asterisk) in a basket-like formation. Note, gephyrin puncta (yellow, arrows) outlining the somato-dendritic membrane. Imaging details (z-thickness in μm , single optical slices unless z-projection type stated): (A) 0.60 μm (B) 0.32 μm (C) 0.36 μm (D) 0.36 μm (E) 0.70 μm , maximum intensity projection (F, left) 33.60 μm , maximum intensity projection (F, right) 0.37 μm .

Chapter 5: Teevra neurons preferentially innervate the CA3 and target axo-axonic cells and CCK-expressing interneurons

5.1 Introduction

Pyramidal place cells in the CA1 and CA3 areas fire rhythmically during theta oscillations and show phase precession relative to dorsal hippocampal CA1 theta oscillations as the animal moves through the place field (O'Keefe and Recce, 1993; Skaggs et al 1996; Maurer and McNaughton, 2007; Huxter et al., 2008; Xu et al., 2015). As a result, they can fire at different phases of individual theta cycles, but the highest number of action potential per theta cycle occurs around the theta LFP trough in the pyramidal cell layer (Fox et al., 1986; Mizuseki et al., 2009). This activity is coordinated by diverse local interneurons of the hippocampus which provide temporally coordinated rhythmic inhibition to distinct subcellular compartments of pyramidal cells (Somogyi et al., 2014), in interaction with the glutamatergic inputs from the entorhinal cortex and the CA3 area. Some of these GABAergic interneurons, e.g. axo-axonic cells (Somogyi et al., 1983) do not fire when the pyramidal cells are most excitable (Viney et al., 2013), whereas others (e.g bistratified and O-LM cells, Katona et al., 2014), which innervate dendrites, fire maximally together with the overall population of pyramidal cells. The way these differences amongst GABAergic interneurons are brought about in the network is beginning to emerge from the analysis of their long-range synaptic inputs (Leao et al., 2012; Fuhmann et al., 2015; Kaifosh et al., 2013, Basu et al., 2016). A key missing link is the knowledge of the theta firing phase preferences of subcortical inputs to defined types of hippocampal interneurons.

Do septo-cortical long-range projecting neurons follow target-region-specific axonal distributions and cell-type-specific theta-phase firing preferences? A striking yet underappreciated feature of GABAergic septal afferents to the hippocampus is the extensive

targeting of interneurons in CA3, the hilus, and granule cell layer of the dentate gyrus (DG) compared to CA1 (Freund and Antal, 1988). This is of particular relevance as the key role of CA3 pyramidal cells in the hippocampal circuit is underlined by their extensive bilateral projections, topographically organised through highly interconnected cell assemblies and providing the numerically largest innervation to both CA3 and CA1 (Witter, 2007). Interestingly, CA3 inactivation does not hamper rate coding in CA1, however it is required for the emergence of theta sequences (Foster and Wilson, 2007; Middleton and McHugh, 2016). Furthermore, the CA3 area and the DG are likely to be involved in distinct aspects of spatial coding (Neunuebel and Knierim, 2014) raising the hypothesis that septal GABAergic inputs to the hippocampal sub-systems might have distinct connectional and temporal organisation. However, the organisation of medial septal inputs to hippocampal or cortical areas at single cell resolution are largely unknown. For example, the degree to which single medial septal GABAergic neurons innervate the CA1, CA3 areas and the dentate gyrus remains unexplored. GABAergic Tevra cells, which have relatively homogenous activity patterns and molecular profiles, provide a unique opportunity to test these questions.

5.2 Experimental setup

In this chapter, I present the anatomical analysis of the axonal arbours of labelled Teevra cells (Table 4.1, Chapter 4). The axonal branches labelled by neurobiotin were visualised and followed either using fluorescence microscopy or conventional light microscopy. Initially, I struggled with the visualisation of the fine collateral branches and terminals of the long-range projecting neurons due to decreasing concentration of neurobiotin in the distal branches. Even the most sensitive method of HRP enzyme reaction following freeze-thaw permeabilisation of the tissue and DAB reaction end product intensification with osmium did not reveal them. Hence, I applied a longer incubation (7 days instead of 2 days) in avidin-biotin-HRP complex, which resulted in a better penetration through the section and more intense visualisation of the finer branches and boutons using light microscopy. I have used this strategy in combination with the most sensitive method of axon visualisation to the full extent of the labelled axon to detect collateral branches.

Using the above technique, I was also able to identify interneurons based on the high levels of mitochondrial endogenous biotin. This is a feature of some, but not all, interneurons. Biotin is a co-factor of many mitochondrial enzymes involved in energy metabolism (e.g. gluconeogenesis) such as, Acetyl-CoA carboxylase, Biotin carboxylase, Propionyl-CoA carboxylase, Pyruvate carboxylase etc., and its high level in PV⁺ cells is in line with their fast firing and high metabolic activity. This granular discontinuous signal of endogenous biotin in mitochondria could be reliably differentiated from the homogeneously filled axon, dendrites, and soma labelled with neurobiotin in the cytoplasm.

The synaptic targets of Teevra cells were tested using immunohistochemistry and sections were processed for revealing up to four molecules in neurons simultaneously. This strategy exploits the fact that different proteins are localised in distinct subcellular compartments (e.g. nucleus,

plasma membrane). I was able to test the same cellular profile in a given section for up to 6 different molecules in sequential reactions of a single section.

In addition to the physiological recorded experimental animals (n = 12 animals from Chapter 4), three mice (n = 2 perfusion fixed by Dr. Katja Hartwich and mouse AJ49) were used as controls to determine the diversity of PV-expressing interneurons in the hippocampal area CA3.

5.3 GABAergic Teevra cells preferentially innervate the CA3 area

Medial septal GABAergic neurons, as a population, innervate all hippocampal regions and many extrahippocampal cortices (Freund and Antal, 1988; Unal et al., 2015), though it is not known if single neurons innervate one or multiple cortical areas. I am unaware of the target area visualisation of single GABAergic septal neurons with known activity patterns in the literature. Accordingly, to explain the basis of the influence of Teevra cells on cortical activity, I tested the distribution of their axonal terminals. All labelled Teevra cells projected either to the left or the right hippocampus. All the labelled Teevra cells whose axon could be followed to branches in the grey matter (n = 11/13) innervated the CA3 region of the hippocampus preferentially and no branches or varicosities were observed in the DG or CA2 (Fig. 5.1C and Fig. 5.2). The axons of Teevra cells travelled to the hippocampus either via the dorsal fornix (n = 2) or the fimbria (n = 11). In CA3, Teevra cells innervated interneurons (Fig. 5.2) which could be identified by high levels of endogenous biotin visualised by the avidin-biotin-HRP complex. Of a total of 472 sampled boutons from 12 coronal hippocampal sections (n = 3 cells, section thickness 70 – 80 μm), 91.5% of boutons were in CA3 (n = 432) and only 8.5% boutons were observed in CA1 (n = 40). The main axon terminated in the hippocampus and no branches were observed in the retrosplenial cortex, the subiculum, the pre- and para-subiculum, or the entorhinal cortex. This preferential termination in CA3 was accompanied by a septo-temporal specialisation of axonal branching with collaterals innervating only a restricted septo-temporal domain in CA3. For single Teevra cells, the majority of axonal branches and boutons were observed only

through 5 to 8 coronal sections (section thickness: 70-80 μm). Although the possibility of incomplete labelling cannot be excluded, most terminal axon collaterals ended in boutons indicating a restricted area of termination. It is unlikely that substantial projections to CA1 were missed. The intrahippocampal spatial positions of the collaterals of the least rhythmic labelled Teevra cell in temporal CA3 (MS11b) did not overlap with those of the most rhythmic neuron (AJ42m) in septal CA3, demonstrating a systematic septo-temporal tiling of the hippocampus.

5.4 Teevra cells target PV⁺ axo-axonic cells as well as CCK⁺ immunopositive interneurons in CA3

As I wanted to determine the synaptic partners of Teevra cells in CA3, it was essential to be able to follow the entire extent of the axon to terminals using fluorescence microscopic visualisation of neurobiotin. The main axon could be followed in most cases, however the branches were faint, making the visualisation of terminal boutons difficult using this method. Therefore, the synaptic targets of Teevra cells in CA3 could only be assessed for two labelled Teevra cells (AJ42m and MS90g, the latter labelled by Mr. Minas Salib). The axon of AJ42m was the most strongly labelled as its collateral branches could be followed to terminal boutons throughout the axonal arbour in CA3, both by fluorescence microscopy and following HRP reaction. The molecular characteristics of 22 cellular target profiles (Table 5.1) were determined. In CA3, 18 out of 22 tested targets were PV⁺, 11 were dendrites and 7 somata. The PV⁺ somatic profiles were all SATB1-immunonegative neurons (Fig. 5.3A, Fig. 5.4). This combination is a strong indicator of axo-axonic cells in the CA1 and CA3 areas (Viney et al., 2013). Four other somatic targets of AJ42m were CCK⁺ cell bodies (Fig. 5.3B) and confirmed to be PV⁻, SATB1⁻, somatostatin⁻ (SST), and calbindin⁻ (CB). Another Teevra neuron, MS90g, was weakly labelled and although the axon and branches could be followed both by fluorescence microscopy and HRP reactions, the terminal boutons were not well resolved in fluorescence microscopy. Triple immunoreactions for PV, SATB1, and CCK in

immunofluorescence were followed first by imaging, then by HRP reaction for bouton visualisation. This strategy helped to identify 6 innervated somata by light microscopy. All targeted neurons were PV⁺ and SATB1-immunonegative (Table 5.1), consistent with the axo-axonic cell molecular combination.

5.5 Estimation of the expected proportion of AACs in the CA3 area and target selectivity of Teevra cells

Viney et al. showed that AACs in the CA1 and CA3 region of the hippocampus were PV⁺ but did not express the transcription factor SATB1. All PV⁺ targets of Teevra cells were consistent with such a profile (Viney et al., 2013). To quantify the potential cell type selectivity of Teevra cells, first the proportion of PV⁺ neurons that are AACs in the CA3 region of mouse hippocampus based on their molecular marker combination was estimated. I performed quadruple immunoreactions for PV, SATB1, SST, and neuropeptide Y (NPY) in three control animals to study the diversity in PV expressing neurons (Fig. 5.5). I have not encountered PV⁺ and NPY⁺ or SST⁺ double-immunopositive cells which do not express SATB1, hence the identity of AACs can be reliably determined using PV and SATB1 immunoreactivity. The proportion of putative AAC cells, PV⁺ neurons not expressing SATB1, in the CA3 was determined to be 30% of the total PV⁺ cells sampled. Therefore, the probability of the observed number of AACs as synaptic targets for single Teevra cells, if all PV⁺ cells were innervated uniformly can be calculated. The probability for AJ42 (7 axo-axonic cell targets found sequentially) is $p = 0.3^7 = 2.1 \times 10^{-4}$, and for MS90g (6 axo-axonic cell targets found sequentially) is $p = 0.3^6 = 7.2 \times 10^{-4}$. I conclude that Teevra cells selectively target AACs amongst PV⁺ neurons of the CA3 hippocampal area, but this does not exclude other interneuron types as some CCK⁺ interneurons were also found as target cells. These CCK⁺ cells were immunonegative for PV⁻, SATB1⁻, SST⁻, and CB⁻. This combination of molecular markers is indicative of CCK basket cells (Lasztóczy et al., 2011) in CA3. Interestingly, both the CCK

basket cells and axo-axonic cells fire action potentials at the peak or ascending phase of dorsal CA1 pyramidal layer theta LFP oscillations (Somogyi et al., 2014), out of phase with the MS Teevra cells firing at the theta LFP trough. Schematic figure summarises the place of Teevra cells in the hippocampal circuit (Fig. 5.6).

5.6 Discussion

Following the discovery that septal GABAergic neurons selectively innervate hippocampal interneurons (Freund and Antal, 1988), it was hypothesised that PV⁺ septal neurons firing at the peak of CA1 theta LFP inhibit both trough firing MS neurons and GABAergic hippocampal neurons that innervate pyramidal cell dendrites in CA1. In turn, theta trough preferring MS neurons were suggested to innervate both the peak firing MS neurons and ‘peri-somatic’ terminating inhibitory cells in CA1 (Borhegyi et al., 2004). Indeed, some trough preferring Teevra cells innervate other PV⁺ MS neurons, but the firing phase of these target cells is unknown. Moreover, none of the theta trough firing rhythmic Teevra cells innervated the CA1 significantly and, instead, targeted PV⁺ axo-axonic cells and CCK⁺ cells in the CA3. The binary phase preference hypothesis (Borhegyi et al., 2004) is also complicated by the fact that both hippocampal GABAergic cells (Klausberger and Somogyi 2008) and theta rhythmically firing GABAergic MS neurons fire at all phases of CA1 theta in non-anaesthetised animals (Dragoi et al., 1999; King et al., 1998; Petsche et al., 1962). The key missing information for explaining this diversity has been the axonal area and target cell preference of any septal neuron with known theta phase firing. The Teevra cells reported here show one example of a sophisticated and highly selective septo-hippocampal synaptic organisation.

Information on the firing phase preference of identified CA3 interneurons is sparse in awake animals. In anaesthetised rats, identified axo-axonic cells in CA3 fired bursts of APs at the peak of dorsal CA1 theta oscillations (Varga et al., 2014; Viney et al., 2013). Axo-axonic cells exclusively innervate the axon initial segment of pyramidal neurons, which is particularly well-

developed in CA3 with up to 150 synapses on a single axon initial segment (Kosaka, 1980). Their action is mediated by GABA_A receptors at the site where the action potential is generated (Buhl et al., 1994) with fast (~ 1.7 ms) inhibitory postsynaptic currents in their synaptic targets recorded at the soma (Ganter et al., 2004). The AACs form synapses with several hundred pyramidal cells at the site where their rhythmic input does not have to be integrated with other inputs on the soma and dendrites. Additionally, since single AACs would influence a large number of pyramidal cells in the same way, they are likely to have a crucial role in rhythmic activity in the hippocampus (Somogyi et al., 1983). Some interneurons expressing CCK, such as CCK basket cells, also fire action potentials at around the peak of CA1 theta oscillations (Lasztóczy et al., 2011). Additionally, amongst the CCK⁺ cells in the CA3 are perirhinal-path-associated cells and CB⁺ cells, both of which are maximally active at the trough of theta oscillations recorded in dorsal CA1 stratum pyramidale (Lasztóczy et al., 2011). The targets of Teevra cell, AJ42m were all CB⁻ immunonegative neurons and hence, are likely to be CCK basket cells, though a definitive molecular combination does not exist. Thus, both identified target cell types of Teevra neurons fire preferentially at the theta peak, counter-phased with the rhythmic input from Teevra cells at the trough of theta (Fig. 5.6). This counter phase firing has been suggested earlier for CA1 (Somogyi et al., 2014) and might indeed be a biological mechanism of theta phase modulation of long-range synaptic partners. These results do not exclude the innervation of various other interneuron types by Teevra cells in CA3, such as PV⁺ basket cells, which fire phase locked to the trough of CA1 theta (Tukker et al., 2013), but coincident firing with and synaptic input from Teevra cells is unlikely.

It is possible that other medial septal GABAergic neuronal types with theta phase preference different from Teevra cells also innervate CA3. Besides a dominant role of septal GABAergic neurons predicted to determine interneuronal firing phase preference, interaction with other rhythmic synaptic inputs, e.g. from the raphe nuclei (Li et al., 2005), supramammillary nucleus

(Vertes., 2015) and from local interneurons and pyramidal cells (Klausberger and Somogyi, 2008) may also contribute to the determination of theta phase firing preference of interneurons. The effects of a potential synaptic influence from Teevra cell innervated CCK-expressing interneurons on axo-axonic cells as suggested for CA1 PV⁺ basket cells (Karson et al., 2009) remains to be tested.

Synaptic inputs to CA1 are temporally organised within theta and gamma timescales. The CA3 pyramidal cell input at the trough and descending phase of CA1 pyramidal layer theta coincides with slow gamma (30 to 80 Hz) oscillations while the medium gamma (60 to 120 Hz) is coupled to the peak of stratum pyramidale theta oscillations (Colgin et al., 2009; Bieri et al., 2014; Fernández-Ruiz et al., 2017; Lasztóczy and Klausberger, 2014; Schomburg et al., 2014). It was previously suggested that MS neuronal firing at gamma intraburst frequencies (30 to 120 Hz) might contribute to these oscillations (Borhegyi et al., 2004; Viney et al., 2013). However, two oscillations occurring at the same frequency might not lead to amplification unless they are phase coupled. Unlike under anaesthesia, MS Teevra neurons in awake animals, on average, had higher intraburst frequencies (upto 300 Hz during RUN) than medium gamma oscillations. The phase coupling of Teevra cell spikes to various gamma oscillations, especially putative CA3 coordinated slow gamma, remains to be investigated with multielectrode arrays to reveal current sources, and was beyond my capacity in this project.

5.7 Conclusion

Teevra cells deliver the same neuroactive substance (GABA) in the same temporal patterns (troughs of theta and LIA) in a brain state-specific manner (do not change their firing rate from REST to RUN significantly) to the same range of synaptic targets (AAC and CCK interneurons in CA3). Therefore, I conclude that Teevra cells are a distinct medial septal cell type. In the next chapter, I study the restricted septo-temporal extent of their axonal arbour in context of the rhythmicity and oscillatory frequency.

Cell ID	Target ID	Target Location	Domain	PV	SATB1	Endo-biotin	CCK	CB	SM	Figure
AJ42m	s47_d1	CA3	d	d+	u	u	u	u	u	5.3B
	s47_d2	CA3	d*	s, d +	n-	s+	s-	s-	s-	
	s47_d3	CA3	d*	s, d +	n+	s+	s-	s-	s-	
	s47_d4	CA3	d*	s, d +	n-	s+	s-	s-	s-	
	s47_d5	CA3	d	d+	u	u	u	u	u	
	s47_s1	CA3	s	s-	s-	s+	s+	s-	s-	
	s47_s2	CA3	s	s-	s-	s+	s+	s-	s-	
	s47_s3	CA3	s	s-	s-	s+	s+	s-	s-	
	s47_s4	CA3	s	s-	s-	s+	s+	s-	s-	
	s48_a1	CA3	s, d	s+	n-	s+	s-	u	u	5.4 B
	s48_a4	CA3	s, d	s+	n-	s+	s-	u	u	5.3A
	s48_a8	CA3	s, d	s+	n-	s+	s-	u	u	5.3A
	s48_a9	CA3	s, d	s+	n-	s+	s-	u	u	5.3A
	s49_a1	CA3	s, d	s+	n-	s+	s-	u	u	5.4 A
	s49_a2	CA3	s, d	s+	n-	s+	s-	u	u	5.4 C
	s50_d1	CA3	d	d+	u	u	u	d-	u	
	s50_d2	CA3	d	d+	u	u	u	d-	u	
	s50_d3	CA3	d	d+	u	u	u	d-	u	
	s50_d4	CA3	d	d+	u	u	u	d-	u	
	s50_d5	CA3	d	d+	u	u	u	d-	u	
	s50_d7	CA3	d	d+	u	u	u	d-	u	
	s50_s1	CA3	s	s+	n-	s+	u	s-	u	
	s71_a10	MS	s	s+	u	s+	u	u	u	
	s71_a11	MS	s	s+	u	s+	u	u	u	
	s71_a9	MS	s, d	s, d +	n+	s+	u	u	u	4.3 D
	s72_a1	MS	s, d	s, d +	n+	s+	u	u	u	
	s72_a2	MS	s, d	s, d +	n+	s+	u	u	u	
	s72_a3	MS	d	d+	u	u	u	u	u	
	s72_a4	MS	d	d+	u	u	u	u	u	
	s72_a5	MS	d	d-	u	u	u	u	u	
	s72_a6	MS	s	s+	n+	s+	u	u	u	
	s72_a7	MS	s	s+	n+	s+	u	u	u	
	s72_a8	MS	d	d+	u	u	u	u	u	
MS90g	s28_s1	CA3	s	s+	n-	s+	s-	u	u	
	s29_s1	CA3	s	s+	n-	s+	s-	u	u	
	s30_s1	CA3	s	s+	n-	s+	s-	u	u	
	s30_s2	CA3	s	s+	n-	s+	s-	u	u	
	s31_s2	CA3	sd	s+	n-	s+	s-	u	u	
	s32_s1	CA3	s	s+	n-	s+	s-	u	u	

Table 5.1. Subcortical and cortical synaptic target neurons of Teevra cells, AJ42m and MS90g

Molecular expression profiles of postsynaptic neurons, based on close apposition of septal terminals. ‘+’ detectable positive immunoreactivity or signal; ‘-’ undetectable immunoreactivity or signal in vicinity of immunopositive signals observed within subcellular domain: s, soma; n, nucleus; d, dendrite; d*, dendrite could be followed to the soma from which it originates; u, unknown (untested or unavailable). MS90g was recorded by Mr. Minas Salib.

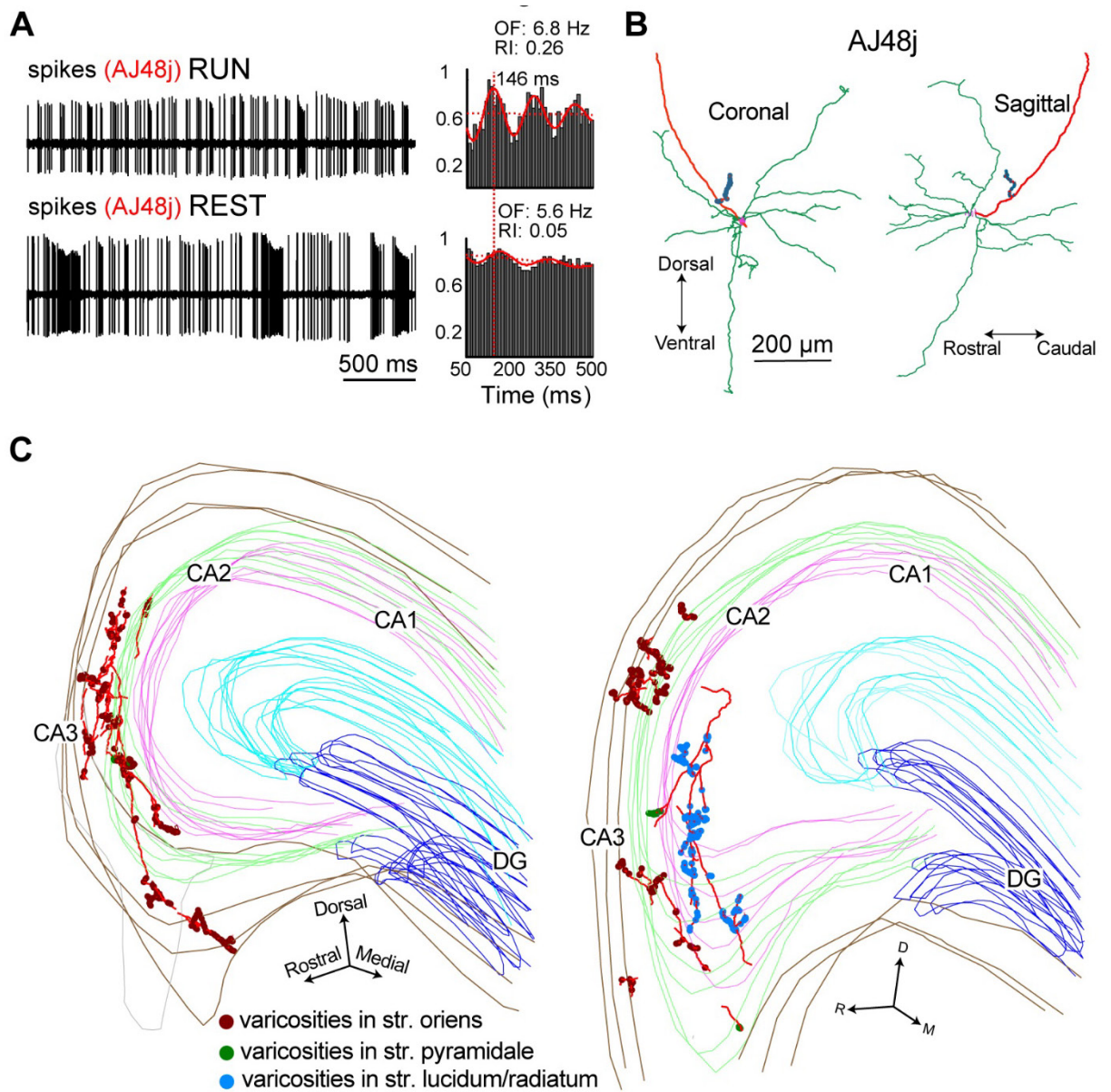


Figure 5.1: Labeled Teevra cell, AJ48j preferentially innervates the CA3 region of the Hippocampus

(A) Activity patterns of labelled Teevra cell AJ48j. Note, the firing rate during REST and RUN does not change significantly during RUN but the rhythmicity index (RI) and oscillatory frequency of firing (OF) increases significantly. (B) Reconstruction of Teevra cell AJ48j in coronal and sagittal views showing a complete ovoid dendritic field (green), soma and main axon (red) and a local axonal branch with boutons (blue). (C) The axonal varicosities (100x objective, colour coded by layer) of AJ48j in two series of consecutive 80 μ m thick sections (left, 4 sections; right, 3 sections), showing preferential termination in part of CA3. Coronal sections are rotated to highlight laminar selectivity (D, dorsal; M, medial; R, rostral). The 3D reconstruction was done by Mr. Michael Howarth.

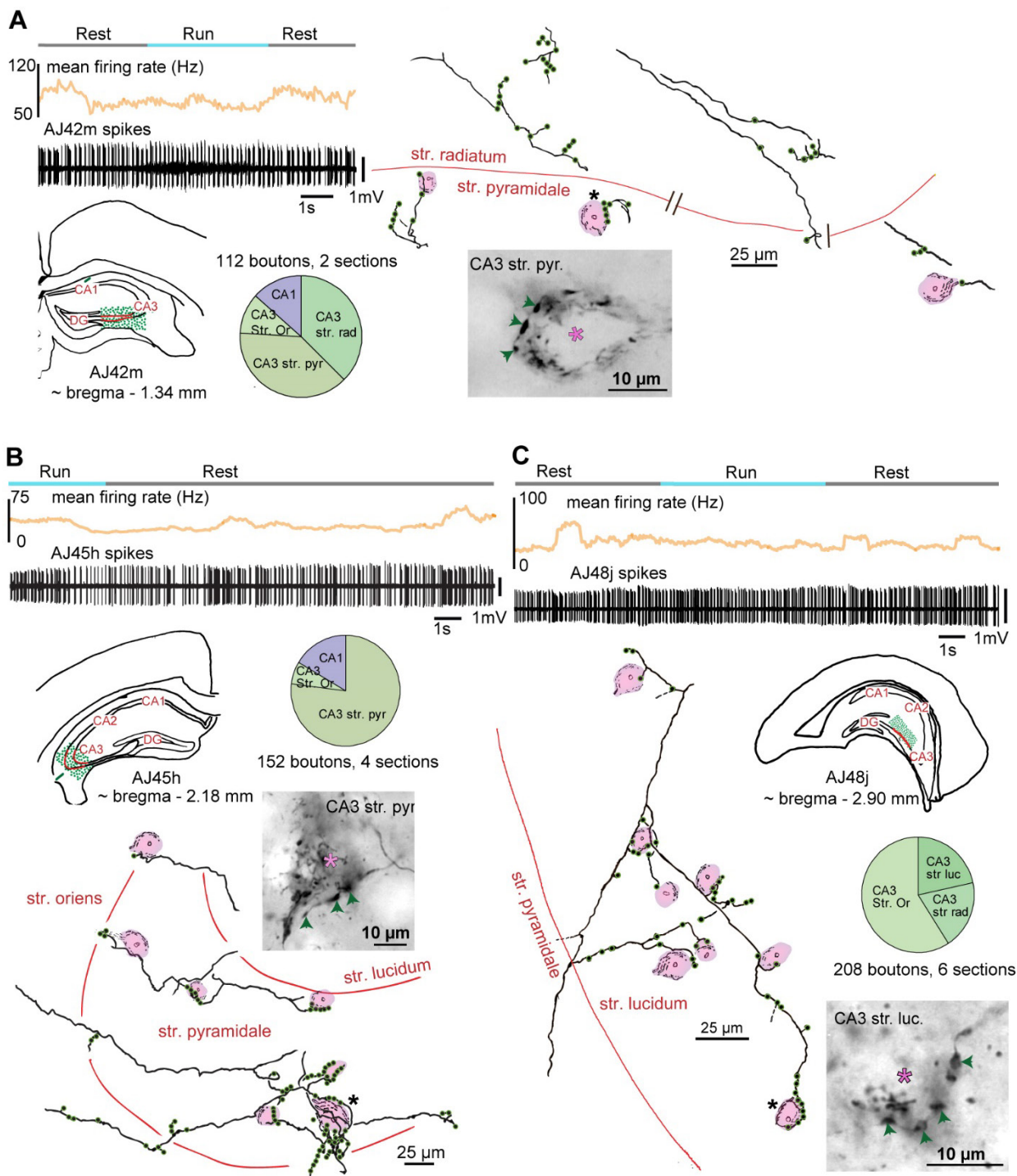


Figure 5.2: Teevra neurons innervate interneurons in spatially restricted domains of CA3

Teevra neurons were identified on the bases of their firing rate change during REST vs RUN and burst duration during CA1 theta (upper panels). Reconstructions of axonal collaterals (green, boutons) of labelled cells reveal that Teevra neurons, AJ42m (**A**), AJ45h (**B**), and AJ48j (**C**) innervate interneurons in the CA3 region. Innervated interneuron somata (shaded pink) are identified by endogenous biotin in mitochondria. Pie charts show representative samples of bouton distribution in different areas and layers. Light micrographs of axonal varicosities (arrows) visualised by HRP enzyme reaction adjacent to cell bodies of individual interneurons (asterisks) rich in endogenous biotin in mitochondria (black in cytoplasm) as revealed by the colour reaction.

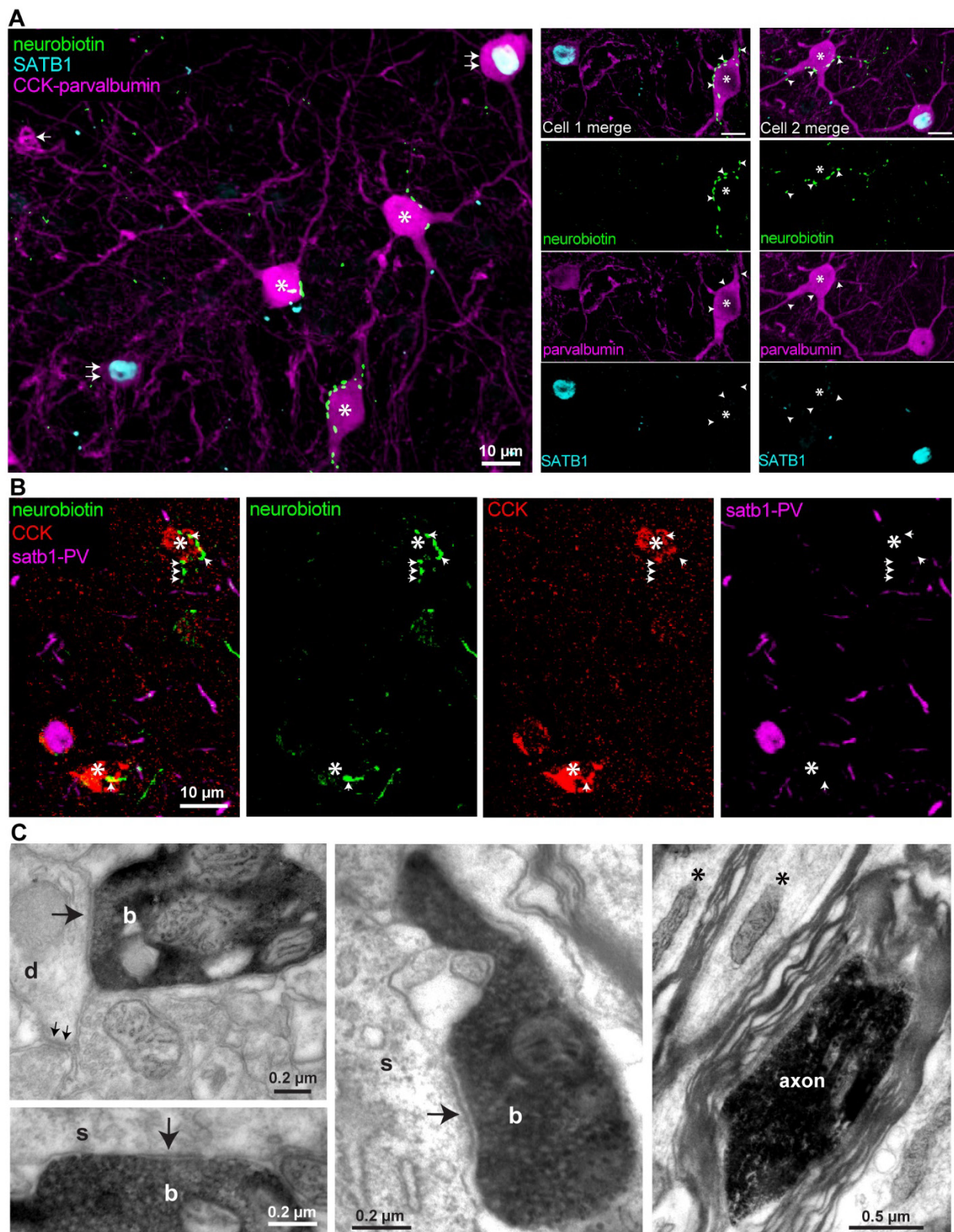


Figure 5.3: Postsynaptic targets of Teevra neurons are PV⁺ or CCK⁺ interneurons

(A) Left, Axonal terminals (green) of Teevra cell AJ42m innervate cells that express PV (magenta, asterisks) but are SATB1-negative (cyan indicates SATB1⁺) cells in a basket-like formation also following their dendrites (merged channels). Nearby PV⁺ and SATB1⁺ cells (double arrows) and a CCK⁺ cell (single arrow) were not innervated. CCK and PV were sequentially reacted and imaged. Right, two innervated cells from left panel channel by channel. (B) Axonal terminals (green, arrows) of Teevra cell AJ42m innervate two interneurons (asterisks) which are CCK⁺ (red), SATB1⁻, and PV⁻ (magenta, sequential reactions) in CA3 str. radiatum. Bottom left of image, non-targeted SATB1⁺ PV-immunonegative neuron. (C) Electron micrographs of Teevra cells AJ42m (left) and AJ45h (middle and right) in CA3. Boutons made type II synaptic junctions (single arrows) with a dendrite (d) and two different somata (s) of interneurons. The postsynaptic dendrite received a type I synapse (double arrows). Right, the main axon of AJ45h is heavily myelinated. Surrounding projection axons (asterisks) in the fimbria have thinner myelin sheaths. Imaging details (z-thickness in μm , z-projection type): (A) left, 34.48 μm standard deviation projection; middle, 9.93 μm standard deviation projection; right, 12.06 μm standard deviation projection; (B) 4.00 μm , average intensity projection. Electron microscopic examination was performed by Prof. Peter Somogyi.

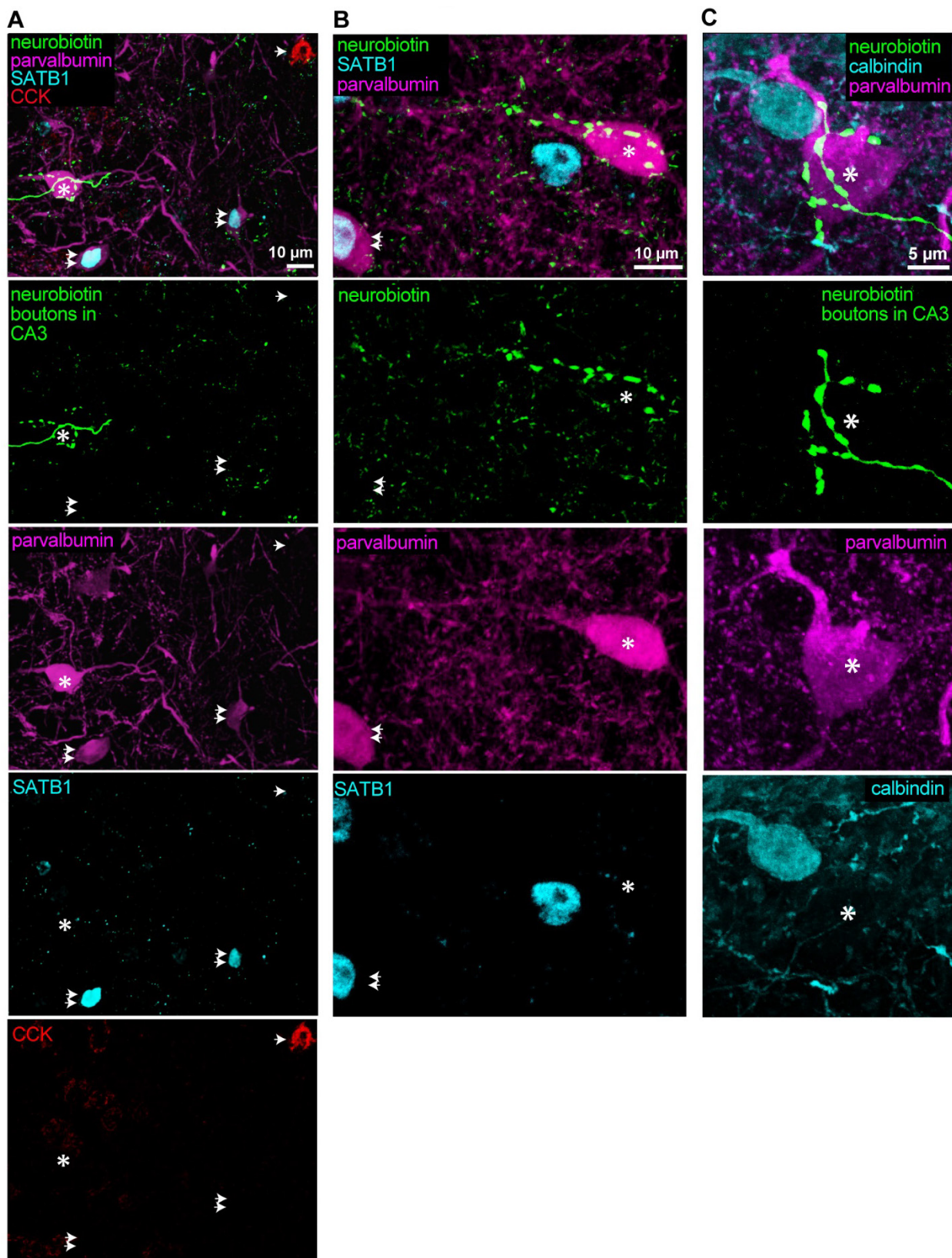


Figure 5.4: Postsynaptic PV⁺/SATB1-negative interneurons are consistently and preferentially innervated by Teevra cells

(A) Axonal terminals (green) of Teevra cell innervate PV⁺ (magenta, asterisks) and SATB1-negative (cyan) cells in a basket-like formation and also following their dendrites. Nearby PV⁺ and SATB1⁺ cells (double arrows) and a CCK⁺ cell (top, single arrow) were not innervated. (B) Axonal terminals (green) of Teevra cell innervate a PV⁺ and SATB1-immunonegative cell in CA1 (labelling as in A). (C) Axonal terminals (green) of Teevra cell in CA3, innervate a PV⁺ (magenta, asterisk) and CB-negative (cyan) cell in a basket-like formation and also follow its dendrite, but do not innervate a neighbouring CB⁺ cell (cyan). The immunoreactivity of targeted interneurons is consistent with the molecular profile of axo-axonic cells. Labels (A), (B) and (C) indicate columns. Imaging details (z-thickness in μm , z-projection type stated): (A) 9.74 μm , standard deviation projection (B) 11.7 μm , standard deviation projection (C) 14.70 μm , maximum intensity projection.

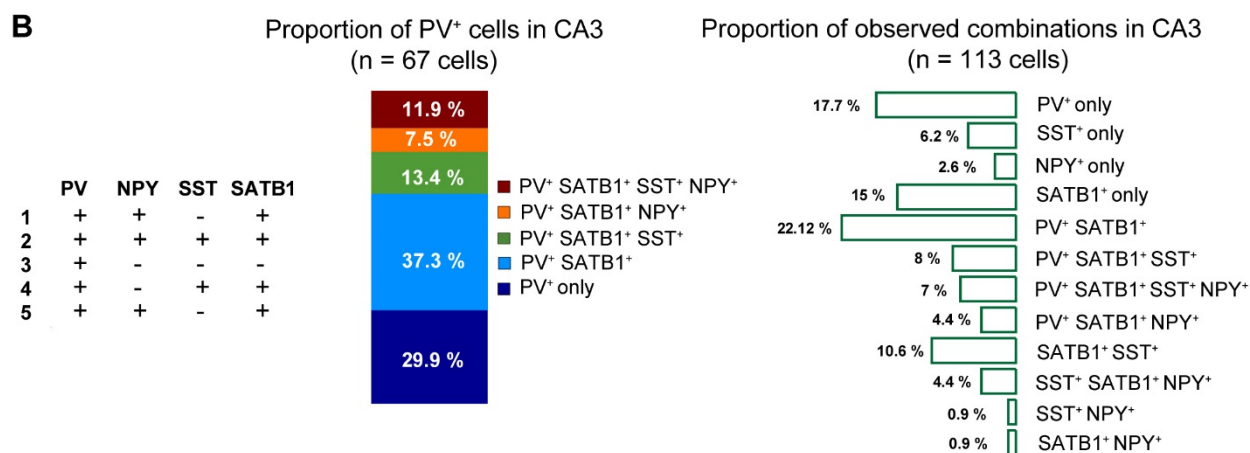
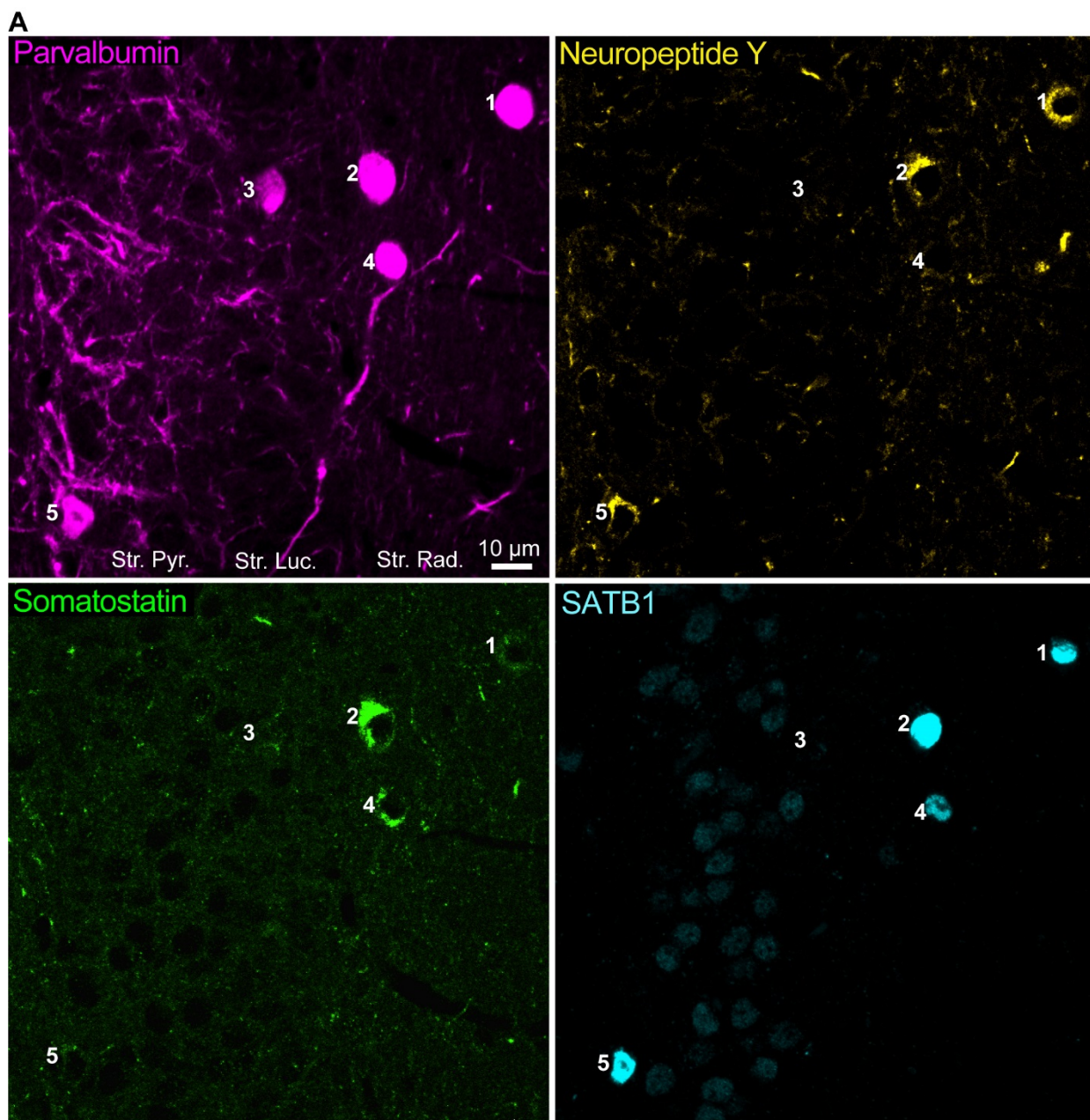


Figure 5.5: Molecular diversity of PV expressing interneurons in mouse CA3

(A) A cluster of PV⁺ interneurons (magenta, 1 to 5) exhibit a diverse combination of immunoreactivity for molecules expressed in strata pyramidale and lucidum of the mouse CA3. The SATB1-immunonegative nucleus of cell 3 is within the displayed optical slice and was also checked in other slices. (B) Left, immunoreactivity scores of the cells in 'A'. Middle, quantification of PV⁺ cells sampled with immunoreactivity for SATB1, NPY, and SST (n = 3 mice, 5 areas). Note that PV-immunopositive and SATB1-immunonegative axo-axonic cells (Viney et al., 2013) comprise 30% of the PV⁺ population. All other PV⁺ cells also are SATB1⁺ in combination with other molecular markers such as SST and NPY. Right, proportions of interneurons labelled for combinations of the 4 molecules. Imaging details (z-thickness): 0.38 μ m, single optical section.

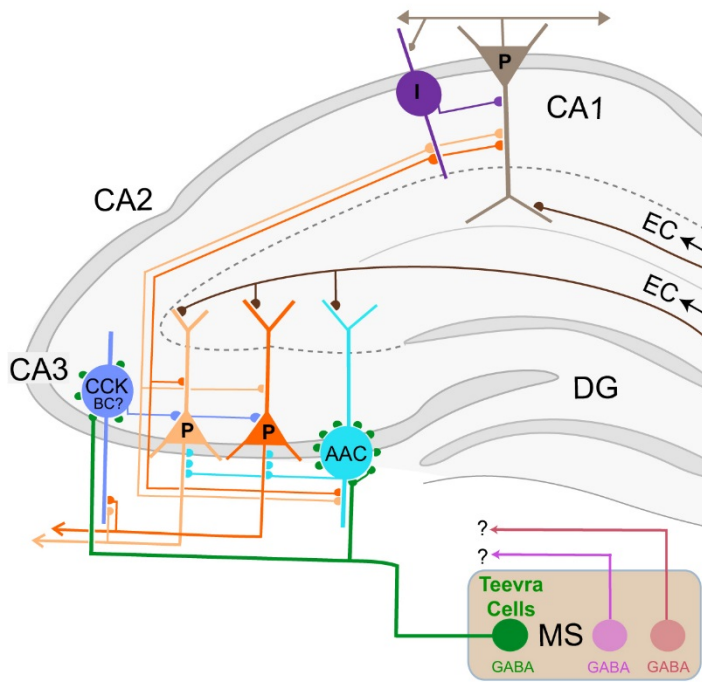
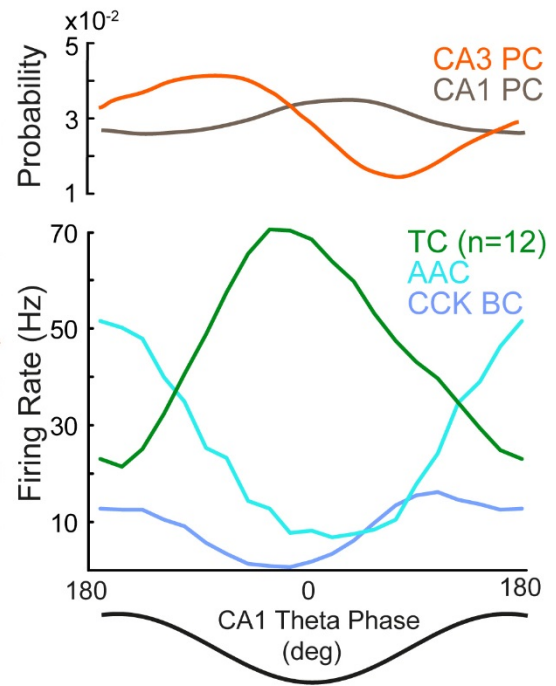
A**B**

Fig 5.6: Theta firing phase selectivity and schematic summary of the place of GABAergic medial septal Teevra cells in the hippocampal circuit.

(A) Teevra cells (green) preferentially innervate CA3 axo-axonic (AAC, cyan) and putative CCK-expressing basket cells (CCK, violet), which in turn target the AIS and somata of CA3 pyramidal cells, respectively. The entorhinal cortical input to CA1 and CA3 innervates pyramidal cells and those GABAergic cells, such as AACs, which have dendrites in the termination zone. Pyramidal cells (P) provide recurrent input to interneurons, to other pyramidal cells and project to other cortical and subcortical areas (arrows). The termination area and target cell selectivity of other GABAergic MS neurons (magenta, brown, and others) remain to be determined. One general GABAergic neuron is shown in CA1 (I, lilac). Dentate granule cells, other types of GABAergic interneurons, cholinergic and glutamatergic MS cells are not shown.

(B) Theta phase relationships of neurons shown in 'A' from recorded data, referenced to dorsal CA1 pyramidal cell layer LFP. On average, Teevra cells ($n = 12$, current study) discharge maximally at the trough of CA1 theta oscillations inhibiting AACs (data from Viney et al., 2013, non-anaesthetised rat, CA1 and CA2 AACs averaged) and putative CCK basket cells (Lasztoczi et al., 2011, anaesthetised rat, CA3) leading to disinhibition of CA3 pyramidal cells, which provide the largest excitatory input to CA1 pyramidal cells (average firing probabilities from Mizuseki et al., 2009). The cell-type-specific temporal modulation of firing rates during theta cycles contributes to the implementation of oscillatory increases and decreases of excitability in pyramidal cell networks via subcellular compartment specific disinhibition.

Chapter 6: Restricted spatio septo-temporal termination of individual Teevra cells in the CA3 area

6.1 Introduction

A prominent feature of Teevra cell axons was the innervation of the CA3 in restricted septo-temporal domains (Chapter 5). The hippocampus can be divided along the longitudinal axis into dorsal, intermediate and ventral parts, which are thought to be integrated into functionally different systems. The longitudinal axis is also referred to as the septo-temporal axis in rodents (Amaral and Witter, 1989) with the dorsal hippocampal region being equivalent to the septal pole (proximity to septal nuclei) and the ventral hippocampus equivalent to the temporal pole (proximity to the temporal lobe). The dorsal and ventral hippocampal regions have distinct connectional architecture (Dolorfo and Amaral, 1998; Swanson and Cowan, 1977; Risold and Swanson, 1996) and there are also differences in molecular marker expression along the septo-temporal axis (Dong et al., 2009). A prominent view is that dorsal hippocampus is associated with spatial navigation and episodic/relational memory (O'Keefe, 1976; Eichenbaum, 1999) and the ventral hippocampus is involved in modulating stress responses and motivational or emotional behaviours (Bannerman et al., 2004; Moser and Moser, 1998). Therefore, these regions have been suggested to be functionally distinct (Fanselow and Dong, 2010).

The most prominent electrophysiological signature of the hippocampal formation are theta oscillations (Buzsáki, 2002) which can be recorded along the entire septo-temporal axis. Recently, it was shown that theta oscillations are 'traveling waves' along the septo-temporal and medio-lateral extent of the hippocampal formation (Lubenov and Siapas, 2009; Patel et al., 2012), such that the phase of theta would shift gradually by 180 degrees across the septo-temporal axis. Theta oscillations are thought to provide temporal windows for local circuit computation (Mizuseki et al., 2009). The maximal input from the CA3 and entorhinal cortex is

at distinct phases (Hasselmo et al., 2002; Mizuseki et al., 2012; Mizuseki et al., 2009). Since the LFP phase across the septo-temporal axis is also representative of local current sinks and sources (Brankačk et al., 1993; Buzsáki et al., 1986), the ‘travelling of theta wave’ might indicate that the ‘current’ time in distinct parts of the hippocampus is distinct in terms of real world time (Lubenov and Siapas, 2009). Additionally, the power of theta oscillations decreases along this axis, reflecting decrease in rhythmicity and theta synchrony of pyramidal cells and interneurons along this axis (Royer et al., 2010). The frequency of theta oscillatory activity does not change significantly (Patel et al., 2012). *How do these differences along the septo-temporal axis emerge?*

As a population, Teevra cells innervated the entire extent of the septo-temporal axis of CA3. In the current chapter, I test the hypothesis that individual Teevra cells innervate distinct septo-temporal regions of the CA3 with distinct phasic, rhythmic or oscillatory input to its target regions.

6.2 Teevra cell location in the septum and the medio-lateral extent of their dendrites

Somato-dendritic reconstructions of Teevra cells were performed to study the medio-lateral and rostro-caudal extents of their dendrites in the MS. Teevra cells have multiple thick, relatively long and non-spiny dendrites originating from the soma. The dendrites branch infrequently in the septum and may cross the septal midline (Fig. 6.1). They spread across 300 - 400 μm medio-laterally and 400 - 500 μm rostro-caudally. The locations of the labelled Teevra cells were close to the septal midline and they were interspersed through the dorso-ventral and rostro-caudal extent of the septum. Location of the somata of Teevra cells relative to the midline of the MS predicted the axonal distribution in the left or right hippocampus respectively as their axons did not cross the midline (Table 4.1).

6.3 Rhythmicity of Teevra cells as a function of their termination along the septo-temporal axis of the hippocampus

As Teevra cells innervated distinct and restricted domains of CA3 along the septo-temporal axis of the hippocampus, I tested whether their rhythmicity index, oscillatory frequency, and firing phase preference showed any correlation with the innervated hippocampal area. I used two measures to estimate the distance along the hippocampus where the first branch or branch with boutons was observed, (1) the linear sagittal distance of the soma section to the section containing the first axonal collateral in the hippocampus as a measure of the anterior-posterior distance from the septum and, (2) using the Scalable Brain Atlas (Bakker et al., 2015). I approximated the medio-lateral (ML), antero-posterior (AP), and dorso-ventral (DV) coordinates of the spatial location in the hippocampus to compute the 3D Euclidian distance of each coordinate from the septal pole of the hippocampal formation (ML : 0; AP: -0.98; DV: -1.4). These distances were highly correlated with each other (linear correlation coefficient: $r = 0.89$, $p = 0.003$). The rhythmicity of the firing of septal neurons strongly decreases the more caudal the termination in the hippocampus (linear correlation coefficient: $r = -0.96$, $p = 0.0001$, $n = 8$ neurons, Fig. 6.2), but the oscillatory burst firing at theta frequency does not change ($p = -0.27$, $n = 8$ neurons, Fig. 6.2). This shows that the depth of modulation of rhythmic GABAergic input to CA3 from the MS decreases the more caudo-ventral the termination in CA3. I have also assessed the mean firing phase distribution of neurons across the septo-temporal axis and found no correlation between these two variables (angular-linear correlation coefficient: $r = 0.38$, $p = 0.57$, $n = 8$ neurons).

6.4 Discussion

The power of theta oscillation decreases along the longitudinal hippocampal axis and the phase of theta shifts by 180° across the septo-temporal extent of the hippocampus (Lubenov and

Siapas, 2009; Patel et al., 2012). Strikingly, I found a strong correlation between the rhythmicity of Teevra neurons and the termination of their axons along the hippocampal longitudinal axis. Highly rhythmic septal Teevra neurons innervate interneurons in the septal pole and less rhythmic neurons innervate interneurons in the temporal pole of the hippocampus, while the oscillatory frequency at which MS input is organised does not change along this axis. This parallels the decrease in theta power and reduction in theta rhythmic neurons in ventral compared to dorsal CA3 (Royer et al., 2010).

Multiple mechanisms have been suggested for the generation of theta waves. One of the suggestions was the existence of a chain of oscillators residing within the septal area and theta waves would be a reflection of the phase delayed septal outputs (Patel et al., 2012). The septo-temporally restricted axons could support such a mechanism. However, I have not found a correlation between the firing phase preference and position of the axon, as our sample of Teevra neurons fired phase coupled to the trough relative to ongoing theta oscillations in the *dorsal* hippocampal CA1. One limitation of these results is the estimation of axonal length. As the hippocampus is curved along the longitudinal axis, it is likely that the relationship of the rhythmicity to the axonal length is not linear. Additionally, the difference in firing phase preference might be small and our small sample size of Teevra cells might not be sufficient to demonstrate a mean phase difference considering the variance and the septo-temporally changing depth of theta modulation of the firing of Teevra cells. These relationships will have to be determined in future investigations by large scale recordings of populations of retrogradely labelled Teevra cells from distinct hippocampal positions. The axons of septal GABAergic neurons are heavily myelinated (Borhegyi et al., 2004) and exhibit high conduction velocities (0.5 – 5 m/s, (Jones et al., 1999)). Thus, the conduction delay is unlikely to contribute to a significant delay of transmission as it is an order of magnitude faster than the propagation velocity (0.16 m/s, (Patel et al., 2012)) of theta waves. However, delays of similar ‘theta’

traveling wave velocity were reported in hippocampal CA3 slices *in vitro* (Miles et al., 1988) while blocking glutamatergic transmission, thus the hippocampal system is capable of generating such a wave through inhibitory connections. *In vivo*, the uniquely positioned Teevra neurons might provide a temporally coherent synchronized GABAergic input, which rhythmically inhibits CA3 interneurons along the hippocampal long axis, thus coordinating pyramidal cell excitability.

As the degree of theta rhythmic modulation of neuronal population activity is of fundamental importance to brain function, the order in Teevra cell theta rhythmicity that I observed, which may transmit to distinct cortical regions differences in rhythmic modulatory input is likely to have biological significance.

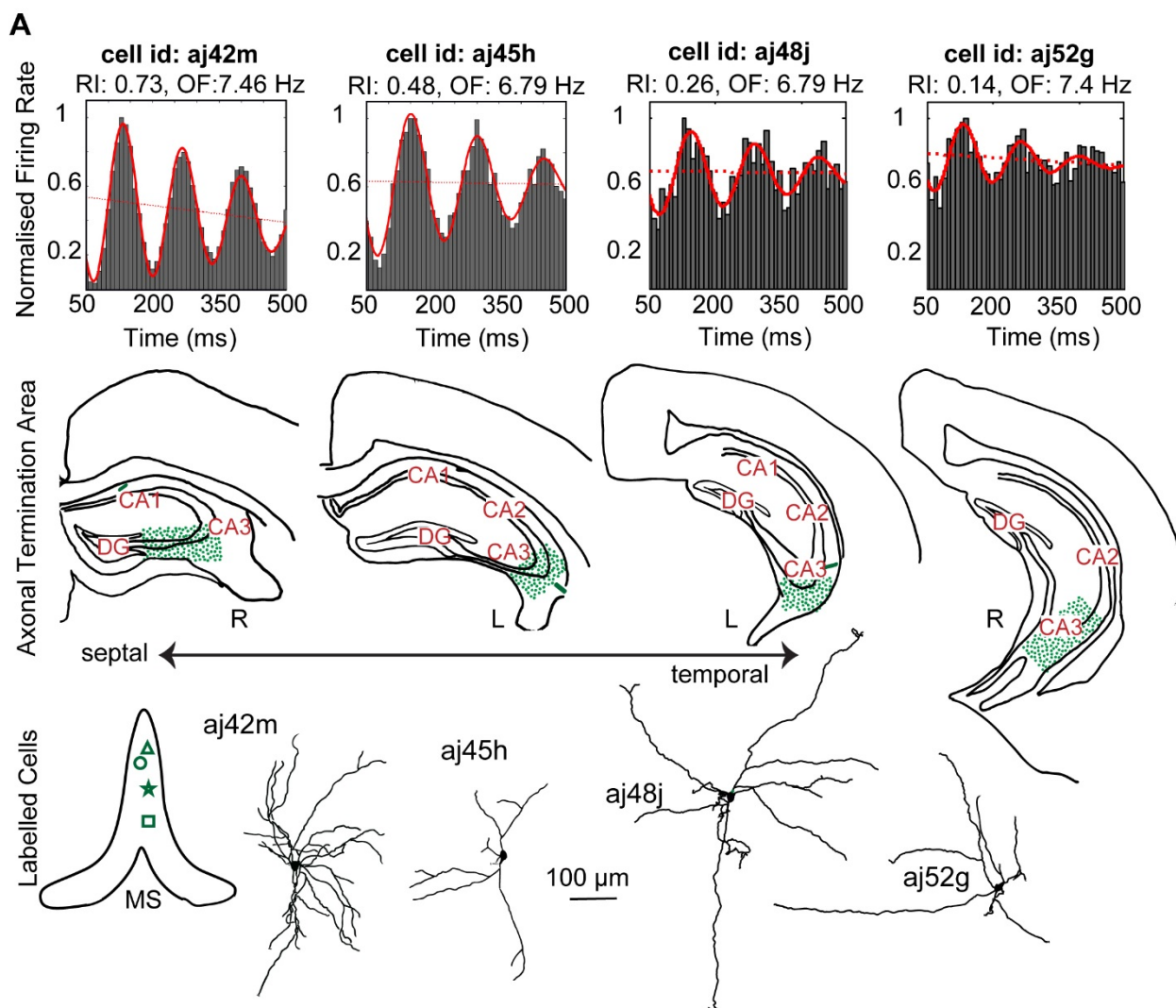


Figure 6.1: Medio-lateral extent of Teevra cell dendrites

(A) Rhythmicity index (RI), oscillatory frequency (OF) and core termination areas (green, dotted) in the CA3 area of individual labelled septal neurons showing that RI decreases the more caudal the termination in CA3; Outlines of the hippocampus (left, L; right, R) were flipped to display them in the same orientation. Bottom, position of cell bodies in the medial septum (projected onto one coronal plane) and reconstructions of their dendritic trees. Teevra cell dendrites spread medio-laterally (range: 300 – 400 μ m), which might influence the organisation of their inputs. I was assisted by Mr. Amar Sharma for the dendritic reconstructions of AJ42m, AJ45h and AJ52g.

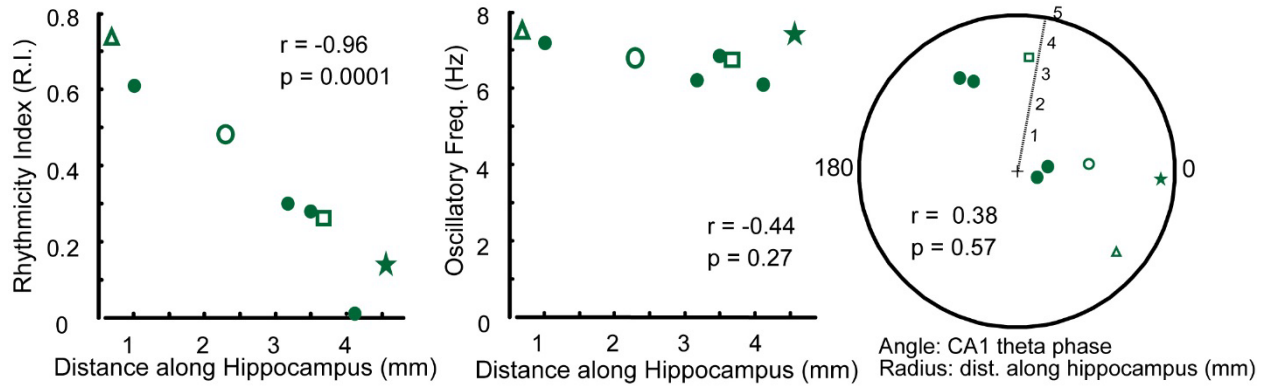
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Figure 6.2: The degree of rhythmicity of Teevra neurons predicts their septo-temporal termination zone in the CA3

(A) Left, Linear negative correlation between the rhythmicity index calculated during RUN and the septo-temporal termination of the axons along the hippocampus ($r = -0.96$, $p = 0.0001$) for labelled Teevra neurons. **Middle,** No correlation appears between the mean oscillatory frequency of firing of the neurons during RUN and the termination of the axons ($p = 0.27$). **Right,** The firing phase preference does not correlate with the distance along the hippocampus ($p = 0.57$).

Chapter 7: Characteristics of Komal cells and other medial septal neurons

7.1 Introduction

In the previous chapters, I characterised the Teevra cells, which are a distinct cell type of neurons projecting from the MS to the CA3 hippocampal area. However, I delineated at least three other populations of rhythmic firing neurons in the MS (Chapter 3) and there exist unclassified non-rhythmic neurons. Further studies are required along the lines of the characterisation of Teevra cells to understand the roles of these populations. The current chapter focuses on information that I obtained from the few examples of extracellularly recorded and juxtacellularly labelled neurons.

The second largest population of rhythmic neurons that I named Komal cells, could be differentiated from Teevra cells based on their action potential burst duration and firing rate change score. Because the Teevra cells formed part of a distinct septo-cortical circuit, I hypothesised that the Komal cells will also have a distinct connectional and molecular architecture. Furthermore, as the Komal cells (Chapter 3) increased their firing rate during RUN periods as compared to REST, their target cells and regions would receive behavioural state (REST vs RUN) and speed inputs from the medial septum as shown for some other neuronal populations (Fuhrmann et al., 2015; Hinman et al., 2016; Justus et al., 2017). In contrast to Komal cells, group 1 neurons decrease their mean firing rate drastically during the running of the animal (Chapter 3). *Do Komal cells and other rhythmic MS cells also project to distinct sub-regions of the hippocampal formation or does the CA3 receive multiple rhythmic inputs from the medial septum?*

7.2 Experimental setup

Komal cells were identified based on their long burst duration and increase in mean firing rate during RUN compared to REST (Video 7.1). The data presented in this chapter are the anatomical analyses of labelled Komal cells ($n = 3$; AJ44j, AJ50j, and AJ50h) and the rare Group 1 cells (AJ45i and AJ19nn, Video 7.2), which decrease their mean firing rate during RUN compared to REST. Two examples of recorded neurons with low rhythmicity (AJ20k and AJ20j) are also shown to demonstrate the diversity of neuronal activity patterns in the MS.

7.3 Physiological, molecular and axonal parameters of labelled Komal cells

I have tested whether the second most populous cluster of rhythmic medial septal GABAergic neurons, the Komal cells ($n = 23$) can be distinguished from Teevra cells based on molecular parameters and target region preference. Komal cells fired bursts of action potentials coupled to the peak of CA1 theta oscillatory activity recorded in stratum pyramidale (Fig. 3.6 and Fig. 3.7). The characteristic feature of Komal cells was an increase in firing rate during the RUN (measured by the rate change score) and positive correlation with the running speed of the animal (Fig. 3.7 and Fig. 7.1). Thus, using the rate change score and long burst duration, individual Komal cells could be differentiated from Teevra cells.

Successfully labelled Komal cells ($n = 3$) were tested for the molecular marker combination expressed by Teevra cells. All three Komal cells were immunopositive for PV, SATB1, and expressed high levels of mGluR1a in their somato-dendritic membrane (Fig. 7.1B and Fig. 7.1C). The latter is in contrast to Teevra cells, in which mGluR1a was not observed at detectable levels. Thus, Teevra and Komal cells could be differentiated based on the expression of mGluR1a in the plasma membrane. This does not exclude the possibility that other cell types may express similar molecular marker combinations. The expression of PV in Komal cells suggests that these are GABAergic neurons. The varicosities of AJ50h were tested, and they

were immunopositive for VGAT. The expression of the vesicular GABA transporter could not be tested in other labelled Komal cells because I did not recover their boutons by fluorescence labelling, due to the low level of injected neurobiotin.

I next tested the axonal termination area of Komal cells by following their axons in HRP reacted sections which provides higher sensitivity than epifluorescence. Komal cell axons projected via the dorsal fornix ($n = 3$). I followed the thick main axons in HRP processed sections and searched for axon collaterals in the hippocampus. None of the Komal cells branched into the hippocampus and instead the main axon carried on to extrahippocampal cortical areas via the dorsal hippocampal commissure (Fig. 7.1D). The axon of AJ50j innervated the retrosplenial cortical area (RSG, Fig. 7.1D) where it targeted interneurons, as indicated by the expression of high levels of endogenous biotin in their mitochondria. The main axon projected more caudally and branches were also observed in the presubiculum. I did not observe branching of the axon of Komal cell AJ50h in RSG, though I found branches and boutons in the presubiculum (Fig. 7.1D). The axon of AJ44j could only be followed to the axon bundle close to the presubiculum and no branching was observed due to weak labelling. Even the most sensitive method of axon visualisation did not reveal its branches suggesting the cell wasn't labelled well enough and neurobiotin did not reach the fine branches. Although I could not visualise the entire axonal arbour of Komal cells, these results show that they projected to extrahippocampal cortical areas bypassing the hippocampal areas CA1, CA2, CA3 or the DG.

7.4 Komal cell activity during LIA

Komal cells were active at the peak of CA1 theta oscillations and increased their firing rate during RUN. I next explored if Komal cells fired action potentials associated to LIA of the LFP during REST. Two of the three recorded Komal cells tested (AJ50j and AJ44j) coupled to the rising phase of the LIA mirroring the coupling to the peak of theta during RUN (Fig. 7.2). Komal cell, AJ50h, did not show any correlation to the large irregular LFP waves. Thus, during

both REST and RUN, some Komal cells are maximally active at the LFP peaks in CA1, coincident with the input arriving to CA1 from the entorhinal cortices (Mizuseki et al., 2009; Schomburg et al., 2014). This is in contrast to the Teevra cell activity, which is maximal at the trough of CA1 LFP, coincident with the pyramidal cell input arriving to CA1 from CA3 (Mizuseki et al., 2009; Schomburg et al., 2014). The autocorrelation histogram of Komal cells during REST periods showed an oscillatory frequency of approximately 5 - 6 Hz.

7.5 Activity patterns of other recorded medial septal neurons

Group-1 cells were rare in my sample of recorded neurons as I observed only four such examples. Either they represent a small population or it is possible that different cell types are concentrated in different parts of the medial septum and diagonal band complex. Their activity patterns were distinct compared to Teevra and Komal cells. Group-1 cells were characterised by a drastic decrease in the firing during RUN compared to REST (Fig. 3.5 and Fig. 7.3A). The location of two labelled cells AJ45i and AJ19nn was more ventral compared to labelled Teevra and Komal cells, though it is difficult to draw conclusions based on few examples. The axon of labelled Group-1 cells were immunonegative for PV, calretinin (CR) and CB. These two cells were not labelled well enough to determine their axonal termination area, though both of the axons could be followed to the fimbria, but did not reach the hippocampal formation (Fig. 7.3).

Another interesting activity pattern I encountered was shown by high firing neurons, which were seemingly 'non-rhythmic'. They had low rhythmicity indices ($RI < 0.1$) and increased their mean firing rate with locomotion (Fig. 7.3C and Fig. 7.3D). The axon of one such labelled cell, AJ20k also projected to extrahippocampal cortex, but its target area could not be determined due to weak labelling. These examples show the diversity of medial septal neuronal firing during distinct behavioural states and suggest that distinct activity patterns might indeed correspond to distinct molecular profiles of neurons and likely different target regions.

7.6 Discussion

Hypothesis on the place of Komal cells in the hippocampo-entorhinal circuit

Teevra and Komal cells are both PV-immunopositive and rhythmically firing, but have distinct burst duration and speed correlation. They can be further differentiated based on the immunoreactivity for mGluR1a on the somato-dendritic membrane. Teevra cells are maximally active at the trough relative to stratum pyramidale theta oscillations and innervate axo-axonic cells in CA3, which are maximally active counter-phased to Teevra cell activity at the peak of theta (Chapter 5). The release of inhibition on the axon initial segment of CA3 pyramidal cells coincides with the maximal firing probability of Teevra cells. Thus, Teevra cell activity is the highest when glutamatergic neurons in their target area, the CA3 pyramidal cells, are most excitable (Fig. 5.6). In contrast, Komal cells are most active at the peak relative to dorsal CA1 theta oscillations recorded in stratum pyramidale. In this chapter, I have shown that Komal cells are PV⁺, extrahippocampal projecting, and target uncharacterised interneurons. One hypothesis is that Komal cells target interneurons in the extrahippocampal cortices, which have a major influence on the firing probability of their target pyramidal cells (e.g. fast spiking axo-axonic or basket cells) thus coordinating the excitatory output from extrahippocampal cortical regions to the hippocampus. As a consequence, the population of neurons being disinhibited would be maximally active coincident with the maximum firing probability of Komal cells, i.e. at the theta peak relative to dorsal CA1 stratum pyramidale. The half theta cycle difference in the maximum firing probability of cortical areas targeted by Komal cells and the CA3 area targeted by Teevra cells would allow local computations to take place before the results are transferred to respective output target regions. It is plausible that medial septal GABAergic Teevra and Komal cells evolved for governing temporal control over the activity of CA1 pyramidal cells by coordinating their two major excitatory inputs, from CA3 and the entorhinal cortex at distinct phases. Entorhinal cortical layer 3 (EC3) neurons are shown to increase their firing probability

at the peak of CA1 stratum pyramidale LFP (Mizuseki et al., 2009; Schomburg et al., 2014) coincident with maximum Komal cell firing and project to the CA1. Unfortunately, no molecular marker combination exists for differentiating PV⁺ basket and other interneurons in the cortex and the interneuron populations are poorly defined. Additionally, the firing phase preference of different types of retrosplenial, presubicular and parasubicular principal neurons are unknown with respect to CA1 theta reference. Preferential labelling of Komal cells in mice that display select interneuron types with genetically introduced markers can help in testing this hypothesis.

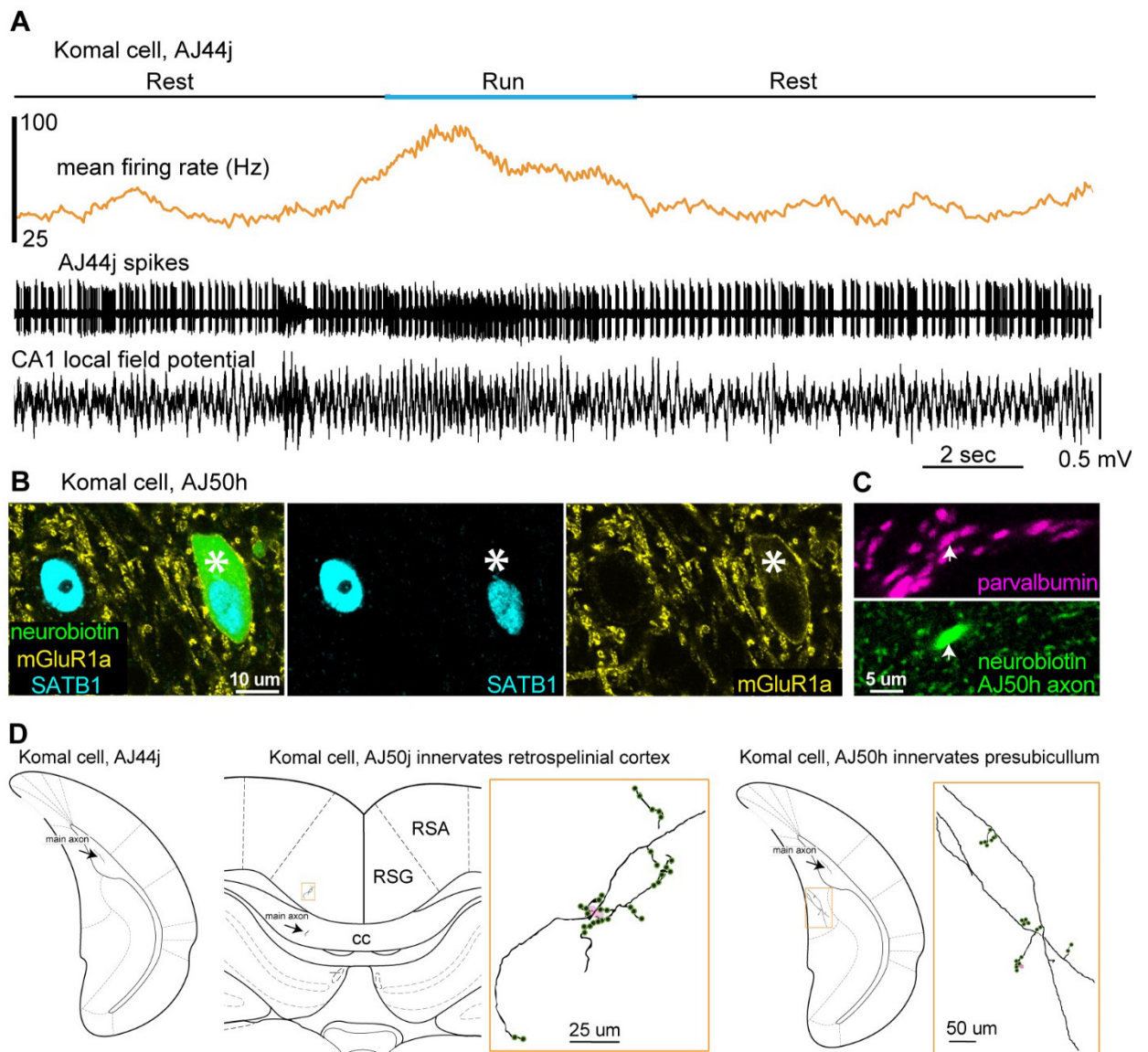


Figure 7.1: Characterisation of identified Komal cells

(A) Komal cell, AJ44j increases its mean firing rate during RUN (blue bar) compared to REST. Note increased rhythmic burst firing during theta oscillatory activity. (B) Juxtacellularly labelled Komal cell, AJ50h (green, asterisk) is immunopositive for SATB1 in its nucleus and expresses mGluR1a on its somatic membrane. Note, nearby SATB1⁺ and mGluR1a⁻ soma. (C) The axon (arrow) of AJ50h is immunopositive for parvalbumin (magenta). (D) The main axon of all three labelled Komal cells: AJ44j (left), AJ50j (middle), and AJ50h (right) project through the dorsal fornix to extrahippocampal cortices. The hippocampus was studied along its rostro-caudal extent and no axon collaterals were observed. AJ50j (middle) innervated the Retrosplenial area (RSA, green boutons) and projected further to the presubiculum. AJ50h (right) innervated the presubiculum. Note, the recovered axonal collaterals were weakly labelled and the full axonal arborisations could not be determined.

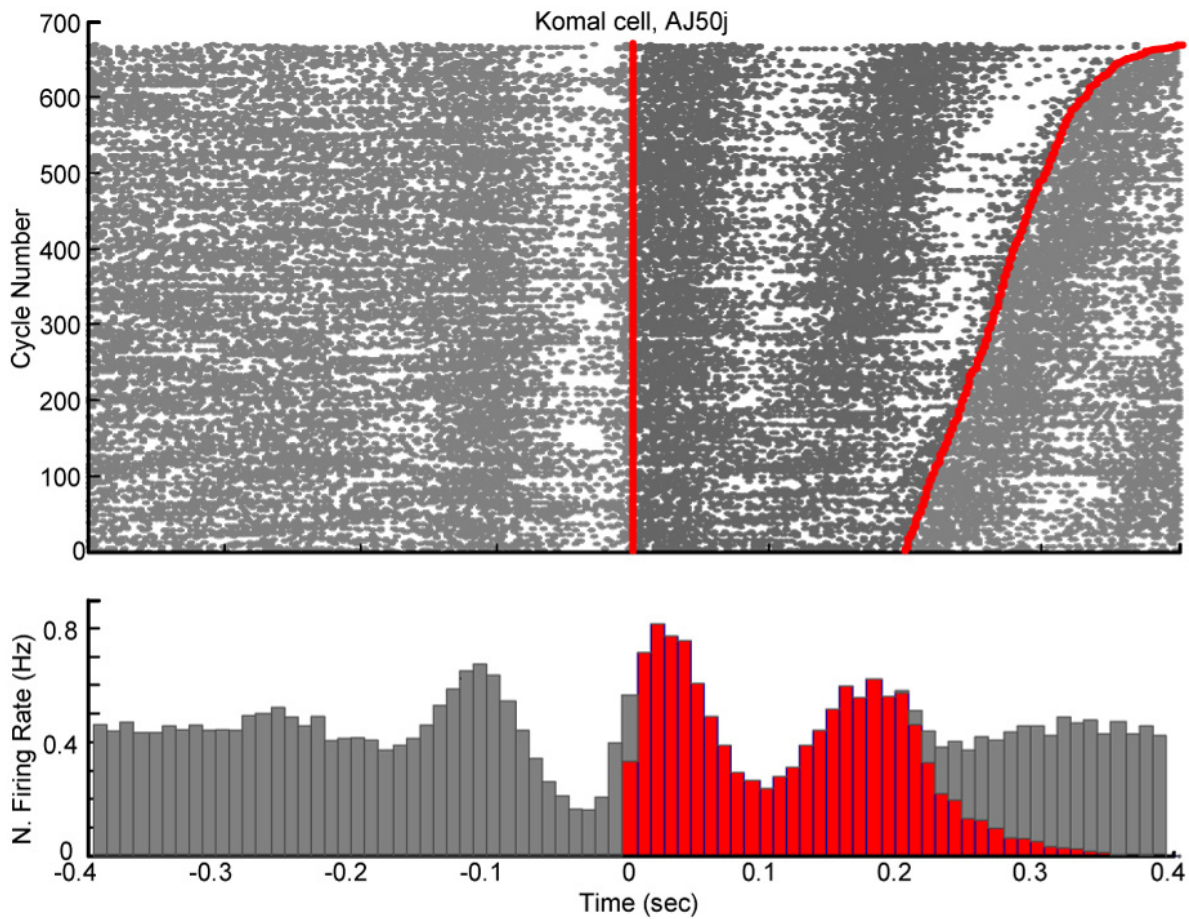


Figure 7.2: The activity pattern of a Komal cell during LIA

Spike raster plot (top) and normalized spike firing histogram (bottom) of Komal cell AJ50j shows correlation of spikes (grey dots) with the timing of slow LFP oscillation cycles. Consecutive LFP zero-crossings of the CA1 LFP (> 200 ms apart) are ordered according to their duration and marked by red lines; time 0 is the zero crossing of LFP rising transitions detected by the algorithm. Note additional burst of action potential at ~ 6 Hz between time points marked by red lines.

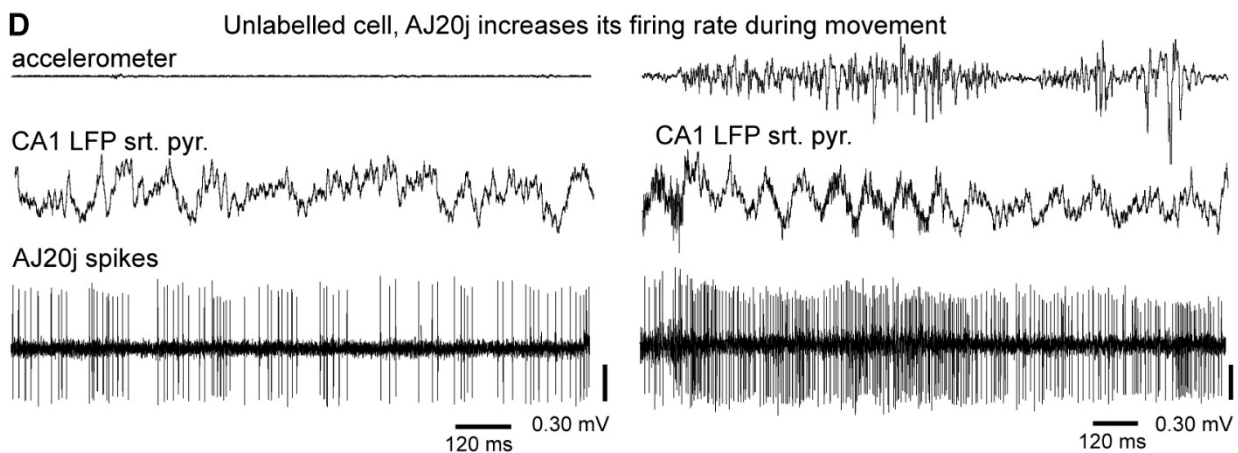
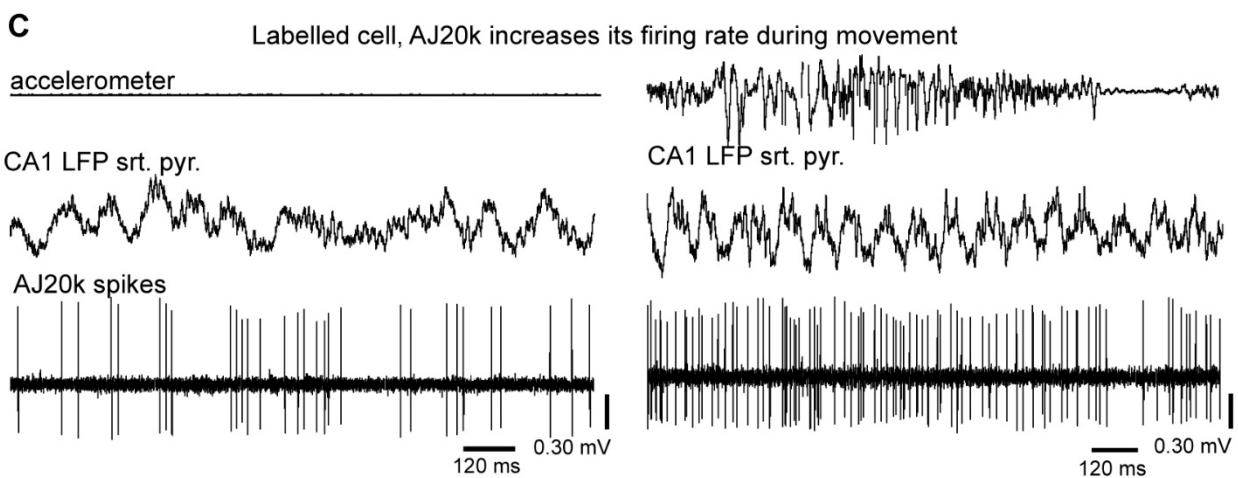
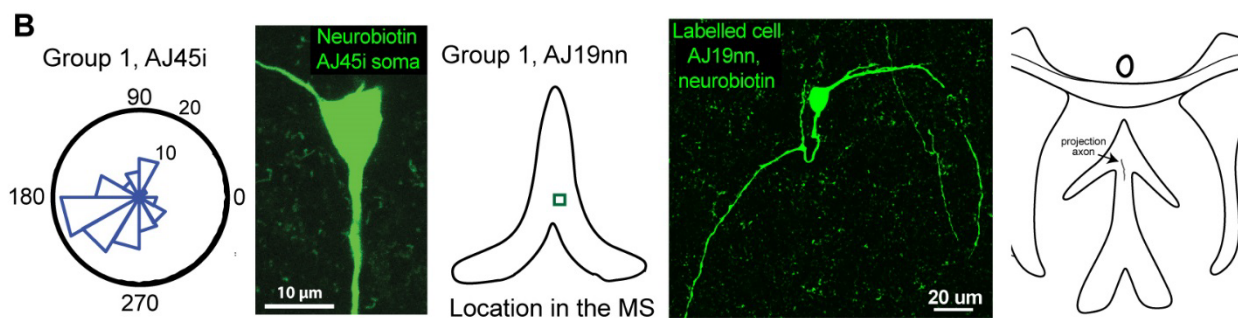
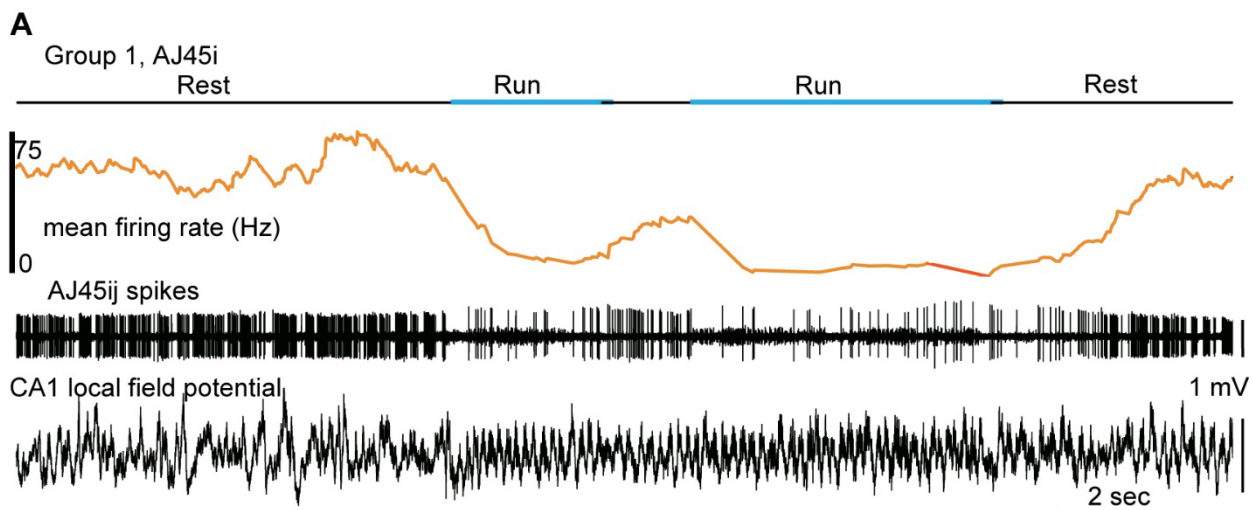


Figure 7.3: Diverse types of medial septal neurons

(A) A labelled neuron in group1, AJ45i decreases its firing rate from REST to RUN. Note, the slight increase in firing during the pause between two RUN epochs. (B) *Left*, cell AJ45i is most active at the peak of hippocampal CA1 stratum pyramidale LFP. *Right*, another neuron in group 1, AJ19nn has dendrites and myelinated projection axon which could be followed to the septal fimbria. (C, D) Medial septal tonic firing neurons, AJ20k and AJ20j increase their firing rate during movement. Their firing patterns are different from Teevra and Komal cells. The target regions of these cells could not be determined due to poor labelling.

Chapter 8: General Discussion

During my studies, I aimed to clarify the contribution of distinct medial septal rhythmic neurons to the coordination of theta oscillatory activity in temporal cortex. I have demonstrated that a medial septal GABAergic cell type that I named the *Teevra* cell, selectively projects to the hippocampal area CA3 and innervates specific types of local GABAergic neuron. This suggests that other regions of the hippocampus and related cortical areas may also receive region and target cell-type-specific subcortical inputs. This hypothesis is supported by the observation that the *Komal* cells also show a restricted termination pattern in extrahippocampal cortices. Such a novel organisational principle endows distinct cell types in the medial septum and diagonal band nuclei with a flexible role in coordinating functionally-related cortical areas, with each parallel pathway adapted to the specific role and requirements of its targets. Teevra cells likely have a special status as they form the largest sub-population of MS rhythmic neurons, at least in the areas where I recorded. Medial septal GABAergic neurons have been hypothesised to be the coordinators of hippocampal theta oscillations (Gogolák et al., 1968; Petsche et al., 1962a; Stumpf et al., 1962). Teevra neurons have a short burst duration, do not significantly change their firing rate from REST to RUN, and fire action potentials at dorsal hippocampal CA1 troughs recorded in stratum pyramidale, coincident with the maximal firing of CA1 pyramidal cells (Mizuseki et al., 2009; O'Keefe and Recce, 1993).

8.1 Teevra and Komal cells coordinate long-range glutamatergic inputs to the CA1

I initially identified Teevra and Komal cells by their activity patterns and subsequently labelled them using the juxtacellular technique. This revealed the axonal termination area of Teevra cells and their synaptic target neurons in CA3. Even though I did not perform a similarly detailed study for Komal cells, I have found that they terminate in extrahippocampal cortices.

The most remarkable feature of Teevra cells is their selective termination in restricted spatial domains along septo-temporal axis of CA3, largely ignoring other hippocampal areas. Overall, my studies show an unexpected differentiation in the spatio-temporal organisation of septo-cortical projection and challenge the assumption that rhythmic septal GABAergic neurons act predominantly via their direct innervation of CA1 interneurons.

Based on my analysis of the synaptic targets of Teevra cells, and assuming that the high frequency bursts fired at the trough of the CA1 theta oscillations lead to inhibition of postsynaptic neurons, I propose that Teevra cells innervate those CA3 interneurons, such as axo-axonic cells and CCK basket cells (Lasztóczy et al., 2011; Somogyi et al., 2014), which preferentially fire around the peak of theta. This would lead to the disinhibition of CA3 pyramidal cell assemblies at the trough of theta (Tóth et al., 1997), which drive pyramidal cell firing in CA1. This temporally coordinated circuit positions Teevra cells as key players in providing precision during retrieval of stored contextual associations around the theta trough (Hasselmo, 2005; Hasselmo et al., 2002; Kunec et al., 2005) and enables the CA3 pyramidal cell output to contribute to temporal coding in the CA1 ensemble (Fernández-Ruiz et al., 2017; Middleton and McHugh, 2016). The synaptic targets of Komal cells could not be determined, but I hypothesise that they are axo-axonic cells or basket cells (FS cells as shown by Gonzalez-Sulser et al., 2014), which are thought to provide effective inhibition of pyramidal cells, thereby coordinating the input from EC3 to CA1. Based on my analysis of the synaptic target regions of Teevra and Komal cells, I suggest that medial septal rhythmic neurons coordinate the timing of converging glutamatergic inputs to CA1 (Kajiwara et al., 2008) from the CA3 and EC3. Lack of this coordination might mediate the loss of theta oscillatory activity in CA1 (Winson, 1978) and impairment in memory (Chrobak et al 1989; McNaughton et al., 2006) during medial septal inactivation.

Place cell representations in the hippocampus could emerge during medial septal inactivation using lidocaine (Brandon et al., 2014; Koenig et al., 2011) though grid cell regularity in the EC was disrupted. Grid cell periodicity was also hampered in another study which used muscimol to inactivate the septum (Brandon et al., 2011). This was accompanied by a loss of rhythmic firing of recorded neurons, though head-direction orientation selectivity was maintained. It is not clear if the new ‘place fields’ that emerge under septal inactivation represent a behaviourally useful place information or an internal default state of the hippocampus to fire at spatial locations. The specific role of the coordination provided by distinct medial septal GABAergic neurons remains to be evaluated in future studies which take into account the temporally phased inhibition by Teevra and Komal cells to distinct parts of the temporal lobe.

8.2 Teevra and Komal cell activity during LIA

During immobility, the hippocampal EEG assumes an apparently irregular pattern called the LIA (O'Keefe, 1978). The most intensively studied feature during LIA are the sharp-wave-ripple oscillations (O'Keefe and Nadel, 1978) but the LIA also exhibits other peaks and troughs, which have been little studied with respect to the activity of septal cells in awake animals. During my studies, I have shown that in addition to being active at the trough of hippocampal theta oscillatory activity as recorded in dorsal CA1, Teevra cell activity is modulated over multiple-timescales, including increased activity at the LIA troughs. In contrast, Komal cells decreased their activity during LIA troughs. *Is the activity of all long-range inputs to CA1 also coupled to distinct phases of the LIA or is this a feature specific to certain septal GABAergic neurons? Do local hippocampal pyramidal cells and interneurons couple their activity to the LIA observed in the hippocampal area CA1?* Both, Teevra and Komal cells fire bursts of action potentials coupled to LIA. I suggest that this coupling may represent a form of hippocampal computation whose temporal windows are determined by Teevra and Komal burst firing. The nature of this computation during immobility remains to be studied in future investigations. It

is plausible that bats use this form of computation through synchronisation mechanisms involving the medial septum, which enables behaviour and learning even in the absence of theta oscillations in the hippocampus (Yartsev et al., 2011).

8.3 Diversity of medial septal neurons and their interconnections

A huge diversity of rhythmic and non-rhythmic activity patterns have been observed in medial septum, both with and without anaesthesia (Dragoi et al., 1999; Gogolák et al., 1968; King et al., 1998; Petsche et al., 1962). In this thesis, I have made an attempt to classify the activity patterns of medial septal neurons based on calculated physiological parameters. Teevra and Komal neurons represent distinct physiological ‘information’ since their spike-trains were modulated differently with a change in the behavioural state of the animal. The choice of the parameters was based on the initial observation that medial septal cellular activity was modulated by running speed (parameter: rate change score), and an attempt to measure the rhythmicity and duration of the burst (parameter: mean burst duration), since these were previously thought to be characteristic of GABAergic medial septal cells. At least four prominent groups of rhythmic MS neurons can now be identified based on their activity patterns in tetrode recordings.

The activity of glutamatergic and cholinergic neurons is not known in awake behaving rodents at single cell resolution, but their contribution to the septo-cortical circuit can be deduced from studies manipulating specific populations using transgenic mouse lines. Recently, optogenetic activation of medial septal glutamatergic cells was suggested to modulate the frequency of theta oscillation at 6 Hz, 9 Hz and 12 Hz, including a corresponding increase in the running speed of the mouse (Fuhrmann et al., 2015). However, glutamatergic medial septal cells are synaptically connected to GABAergic medial septal cells (Leao et al., 2015). So the effects of the stimulation cannot be attributed to the effects of glutamatergic cells directly on hippocampal cells during locomotion. Interestingly this result could not be replicated by stimulations of glutamatergic

fibres (Robinson et al., 2016) in the fornix, suggesting that the effect observed by Fuhrmann et al., (Fuhrmann et al., 2015) might be due to intraseptal connections. It is plausible that Komal cells are targets of medial septal glutamatergic neurons, whose activity patterns with respect to theta oscillations are not known in awake animals. Glutamatergic MS cells might increase their firing rate with movement, since their calcium transients in their boutons increased with locomotion (Justus et al., 2017). Additionally, speed associated activity changes are not restricted to medial septal neurons. Both, afferent and efferent connections of the septum are known to exhibit behavioural state related activity patterns including ‘speed’ signals. Some examples include, pyramidal cells and interneurons in the CA1 (Czurkó et al., 2011; Varga et al., 2012; Varga et al., 2014), interneurons in the medial entorhinal cortex (Hinman et al., 2016; Kropff et al., 2015), neurons of the visual cortex (Saleem et al., 2013), and in the mesencephalic locomotor region (Roseberry et al., 2016). It is likely that most parts of the brain represent some aspect of the behavioural state of the animal. Hence, the contribution of medial septal glutamatergic, GABAergic, and cholinergic neurons needs to be investigated at a single cell resolution in awake animals.

Both Teevra and Komal cells were immunopositive for parvalbumin and SATB1, but differed in the expression of mGluR1a. I have observed PV⁺ cells which are not consistent with the molecular marker combinations that define Teevra and Komal cells (e.g. PV⁺/SATB1⁻/mGluR1⁻). This suggests that other PV⁺ cells with unknown firing phase preference and rhythmicity also exist in the MS. Understanding these distinct cell types and their organisational structure will be crucial to explain how the MS transmits rhythmic signals from the brainstem theta rhythm generators (Vertes and Kocsis, 1997). These diverse medial septal cell types, even within the PV⁺ subpopulation, should also be taken into account in studies using optogenetic manipulations in the septo-hippocampal system. Stimulating PV⁺ medial septal neurons at the same time would synchronously activate all septal PV-expressing cells at the same phase with

respect to theta, even though they have diverse firing phase preferences and synaptic targets in the non-manipulated network. An alternative preparation might be developed in order to integrate the phase and target region information into the experiment, stimulating PV septal axons of Teevra cells in CA3 at the trough and of Komal cells in EC3 at the peak relative to theta oscillations recorded in dorsal CA1.

8.4 Do medial septal neurons coordinate hippocampal theta oscillations by modulating the firing of their synaptic targets?

Across distinct brain regions, long-range GABAergic connections are responsible for temporal coordination (Basu et al., 2013; Basu et al., 2016; Jinno et al., 2007; Melzer et al., 2012) and are thought to transmit oscillatory activity in collaboration with local GABAergic circuits through reciprocal inhibitory connections. A breakdown in the long-range temporal connections and/or in the regulation of inhibition is a hallmark of various malfunctions of the central nervous system, some of which include abnormal electrical discharge or seizure (de Lanerolle et al., 1989), auditory verbal hallucination in schizophrenia (Koutsoukos et al., 2013), and major depression (Linkenkaer-Hansen et al., 2005). If theta oscillatory cycles provide temporal windows of synchrony for circuit computation, aberration of theta oscillatory activity may cause the behavioural deficits observed. *What proportion of the activity of cortical GABAergic neurons is determined by their long-range GABAergic synaptic inputs?* There were two main challenges in addressing this question. First, the activity patterns of defined cortical GABAergic cell types and their inputs were not known in theta oscillatory timescales (80 to 150 ms) and second, the technical difficulties of labelling long-range GABAergic projection axons with known physiological parameters at single cell resolution. It was fortunate that the main cell type that I characterised, the Teevra cells, projected to interneurons in the CA3 region of the hippocampus. The firing phase preference of some cell types in the CA3 and molecular marker combinations to identify them have been described (Lasztóczy et al., 2010; Tukker et al., 2013;

Viney et al., 2013). In the thesis, I have reported the molecular identity of the targets of two Teevra cells (cell ID: AJ42m, MS90g), both of which preferentially innervated axo-axonic cells amongst the diverse PV⁺ interneuron population. This is interesting because single AACs influence a large number of pyramidal cells at the site of action potential generation and are thereby likely to have a major role in controlling rhythmic activity in the hippocampus. Additionally, this result provides evidence that theta-trough firing Teevra cells innervate theta peak firing AACs (Viney et al., 2013). This counter phase firing has been suggested earlier for CA1 (Somogyi et al., 2014) and I propose this to be a mechanism for the phasic inhibition of those populations of cortical neurons, whose synchronisation to the brainstem ‘theta’ generating network is critical to shape inhibition across distinct cortical regions. Even though, my results do not exclude the innervation of various other interneuron types by Teevra cells in CA3, it is unlikely that Teevra cells fire coincident with their synaptic target neurons. Besides a dominant role of septal GABAergic neurons predicted to determine the firing phase preference of interneurons, interaction with other rhythmic synaptic inputs, e.g. from the raphe nuclei (Li et al., 2005), supramammillary nucleus (Vertes., 2015), and from local interneurons and pyramidal cells (Klausberger and Somogyi, 2008) may also contribute to the determination of theta phase firing preference. Exploring the relative contributions of these inputs is necessary to determine the neurological and behavioural loss during neurodegeneration attributable to each input.

8.5 Comparison of the Teevra and Komal cell activity with previous recordings of septal neurons

The current study expands on a variety of criteria that have been proposed for the classification of medial septal cells (Petsche et al., 1962; Gaztelu & Buno, 1982; Alonso et al., 1987; King et al., 1998; Ford et al., 1989). Most previous studies used rhythmicity of the autocorrelation histogram as a basic classifier, which I have also found to be very useful as a distinguishing

feature for rhythmic and apparently ‘non-rhythmic’ medial septal cells. Previous studies have also observed differences in burst duration (King et al., 1988; Borhegyi et al., 2004). Theta related activity (theta-on and theta-off) was also used as a classifier in urethane anaesthetised rats (Ford et al., 1989), however this parameter does not directly relate to the rate change score that I have used. This behavioural- and network- state difference is crucial since it was observed that under urethane anaesthesia, the number of rhythmically bursting cells increases significantly over the unanaesthetised state (Lee et al., 1991). Thus it is not possible to ‘truly’ match the previously recorded medial septal cells according to the clustering criteria that I have used, even though similar diversity of septal neuronal activity patterns have been observed before. In this section, I briefly discuss the similarities between the results obtained from unsupervised clustering used in this thesis to the grouping used in previous studies.

King et al (1998) made recordings in awake rats and characterised neurons into three groups. Amongst them, type-1 cells showed rhythmicity in their autocorrelograms and type-2 and type-3 did not show rhythmic autocorrelograms. I have also observed rhythmic and non-rhythmic medial septal cells that are illustrated in this thesis (Chapters 3 and 7). Type-1b cells (King et al., 1998) show Teevra cell like firing, with highly rhythmic autocorrelations and short burst durations and Type-1a cells (King et al., 1998) show Komal cell like firing, with rhythmic autocorrelations and long sustained bursts. Borhegyi et al (2004) also suggested that short and long burst duration neurons have distinct firing phase preferences under anaesthesia. Interestingly, the short burst duration neurons and long burst duration neurons preferred opposite phases with respect to ongoing theta oscillations recorded in stratum pyramidale, consistent with the Teevra cell firing at the trough and Komal cell firing at the peak. Thus various medial septal cells which I have identified have been recorded previously, however they couldn’t be grouped into cell types due to the absence of a parameter similar to the rate change score and lack of knowledge of their synaptic targets.

8.6 Outlook

During my DPhil, I have used congruent neuronal features to define a previously undescribed organisation of the septo-hippocampal GABAergic pathway. The results demonstrate the cellular diversity in the medial septal nucleus and provide a spatio-temporal framework for understanding the long-range, parallel subcortical innervation, which coordinates network oscillations in the cortex via local inhibitory neurons. Cortical region-specific GABAergic innervation by physiologically distinct septal neuronal types might indicate a general mechanism for the coordination of network activity, both during regular theta oscillations and irregular large amplitude waves, providing windows of computation through synchronisation.

References

- Airaksinen, M.S., Eilers, J., Garaschuk, O., Thoenen, H., Konnerth, A., and Meyer, M. (1997). Ataxia and altered dendritic calcium signaling in mice carrying a targeted null mutation of the calbindin D28k gene. *Proc Nat Acad Sci USA* 94, 1488-1493.
- Alonso, A., Gaztelu, J.M., Buno, W., and García-Austt, E. (1987). Cross-correlation analysis of septohippocampal neurons during theta-rhythm. *Brain Research* 413, 135-146.
- Amaral, D.G., and Witter, M.P. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* 31, 571-591.
- Bakker, R., Tiesinga, P., and Kötter, R. (2015). The Scalable Brain Atlas: Instant Web-Based Access to Public Brain Atlases and Related Content. *Neuroinformatics* 13, 353-366.
- Bannerman, D.M., Rawlins, J.N., McHugh, S.B., Deacon, R.M., Yee, B.K., Bast, T., Zhang, W.N.N., Pothuizen, H.H., and Feldon, J. (2004). Regional dissociations within the hippocampus--memory and anxiety. *Neurosci. Biobehav. Rev.* 28, 273-283.
- Basu, J., Srinivas, K.V., Cheung, S.K., Taniguchi, H., Huang, Z.J., and Siegelbaum, S.A. (2013). A cortico-hippocampal learning rule shapes inhibitory microcircuit activity to enhance hippocampal information flow. *Neuron* 79, 1208-1221.
- Basu, J., Zaremba, J.D., Cheung, S.K., Hitti, F.L., Zemelman, B.V., Losonczy, A., and Siegelbaum, S.A. (2016). Gating of hippocampal activity, plasticity, and memory by entorhinal cortex long-range inhibition. *Science* 351, aaa5694.
- Bennett, M.R., Gibson, W.G., and Robinson, J. (1994). Dynamics of the CA3 pyramidal neuron autoassociative memory network in the hippocampus. *Philosophical Transactions: Biological Sciences*, 167-187.
- Berry, S.D., and Thompson, R.F. (1979). Medial septal lesions retard classical conditioning of the nictitating membrane response in rabbits. *Science* 205, 209-210.
- Bieri, K.W., Bobbitt, K.N., and Colgin, L.L. (2014). Slow and fast γ rhythms coordinate different spatial coding modes in hippocampal place cells. *Neuron* 82, 670-681.
- Borhegyi, Z., Varga, V., Szilagy, N., Fábó, D., and Freund, T.F. (2004). Phase segregation of medial septal GABAergic neurons during hippocampal theta activity. *J. Neurosci.* 24, 8470-8479.
- Brandon, M.P., Bogaard, A.R., Libby, C.P., Connerney, M.A., Gupta, K., and Hasselmo, M.E. (2011). Reduction of theta rhythm dissociates grid cell spatial periodicity from directional tuning. *Science* 332, 595-599.
- Brandon, M.P., Koenig, J., Leutgeb, J.K., and Leutgeb, S. (2014). New and distinct hippocampal place codes are generated in a new environment during septal inactivation. *Neuron* 82, 789-796.
- Brankack, J., Stewart, M., and Fox, S.E. (1993). Current source density analysis of the hippocampal theta rhythm: associated sustained potentials and candidate synaptic generators. *Brain Res* 615, 310-327.
- Brioni, J.D., Decker, M.W., Gamboa, L.P., Izquierdo, I., and McGaugh, J.L. (1990). Muscimol injections in the medial septum impair spatial learning. *Brain Research* 522, 227-234.

Bunce, J.G., Sabolek, H.R., and Chrobak, J.J. (2004). Intraseptal infusion of the cholinergic agonist carbachol impairs delayed-non-match-to-sample radial arm maze performance in the rat. *Hippocampus* 14, 450-459.

Buzsaki, G. (2002). Theta oscillations in the hippocampus. *Neuron* 33, 325-340.

Buzsáki, G. (1986). Hippocampal sharp waves: their origin and significance. *Brain Res* 398, 242-252.

Buzsaki, G., Czopf, J., Kondakor, I., and Kellenyi, L. (1986). Laminar distribution of hippocampal rhythmic slow activity (RSA) in the behaving rat: current-source density analysis, effects of urethane and atropine. *Brain Res* 365, 125-137.

Buzsaki, G., and Draguhn, A. (2004). Neuronal oscillations in cortical networks. *Science* 304, 1926-1929.

Calandreau, L., Jaffard, R., and Desmedt, A. (2007). Dissociated roles for the lateral and medial septum in elemental and contextual fear conditioning. *Learning & Memory* 14, 422–429.

Cape, E.G., Manns, I.D., Alonso, A., Beaudet, A., and Jones, B.E. (2000). Neurotensin-induced bursting of cholinergic basal forebrain neurons promotes g and t cortical activity together with waking and paradoxical sleep. *J. Neurosci.* 20, 8452-8461.

Carpenter, F., Burgess, N., and Barry, C. (2017). Modulating medial septal cholinergic activity reduces medial entorhinal theta frequency without affecting speed or grid coding. *Scientific Reports* 7, 14573.

Chrobak, J.J., Stackman, R.W., and Walsh, T.J. (1989). Intraseptal administration of muscimol produces dose-dependent memory impairments in the rat. *Behav Neural Biol* 52, 357-369.

Cobb, S.R., Buhl, E.H., Halasy, K., Paulsen, O., and Somogyi, P. (1995). GABAergic interneurons can pace rat hippocampal principal cell activity through perisomatic synapses in vitro. *J. Physiol. (Lond)* 487, 53P.

Colgin, L.L., Denninger, T., Fyhn, M., Hafting, T., Bonnevie, T., Jensen, O., Moser, M.B., and Moser, E.I. (2009). Frequency of gamma oscillations routes flow of information in the hippocampus. *Nature* 462, 353-357.

Colom, L., Castaneda, M., Reyna, T., Hernandez, S., and Garrido-Sanabria, E. (2005). Characterization of medial septal glutamatergic neurons and their projection to the hippocampus. *Synapse* 58, 151-164.

Csicsvari, J., Hirase, H., Czurkó, A., Mamiya, A., and Buzsáki, G. (1999). Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. *J. Neurosci.* 19, 274-287.

Czurkó, A., Huxter, J., Li, Y., and Hangya, B. (2011). Theta phase classification of interneurons in the hippocampal formation of freely moving rats. *J. Neurosci.* 31, 2938-2947.

de Lanerolle, N.C., Kim, J.H., Robbins, R.J., and Spencer, D.D. (1989). Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res* 495, 387-395.

Dolorfo, C.L., and Amaral, D.G. (1998). Entorhinal cortex of the rat: topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. *J. Comp. Neurol.* 398, 25-48.

- Dong, H.-W.W., Swanson, L.W., Chen, L., Fanselow, M.S., and Toga, A.W. (2009). Genomic-anatomic evidence for distinct functional domains in hippocampal field CA1. *Proc Nat Acad Sci USA* *106*, 11794-11799.
- Douchamps, V., Jeewajee, A., Blundell, P., Burgess, N., and Lever, C. (2013). Evidence for encoding versus retrieval scheduling in the hippocampus by theta phase and acetylcholine. *J. Neurosci.* *33*, 8689-8704.
- Dragoi, G., Carpi, D., Recce, M., Csicsvari, J., and Buzsáki, G. (1999). Interactions between hippocampus and medial septum during sharp waves and theta oscillation in the behaving rat. *J. Neurosci.* *19*, 6191-6199.
- Eichenbaum, H. (2014). Time cells in the hippocampus: a new dimension for mapping memories. *Nat Rev Neurosci* *15*, 732-744.
- Fanselow, M.S., and Dong, H.W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures. *Neuron* *14*, 7 - 19.
- Fernández-Ruiz, A., Oliva, A., Nagy, G.A., Maurer, A.P., Berenyi, A., and Buzsáki, G. (2017). Entorhinal-CA3 dual-input control of spike timing in the hippocampus by theta-gamma coupling. *Neuron* *93*, 1213-1226.
- Ferraguti, F., Cobden, P., Pollard, M., and Cope, D. (2004). Immunolocalization of metabotropic glutamate receptor 1 α (mGluR1 α) in distinct classes of interneuron in the CA1 region of the rat hippocampus. *Hippocampus* *14*, 193-215.
- Floresco, S.B., Todd, C.L., and Grace, A.A. (2001). Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. *J. Neurosci.* *21*, 4915-4922.
- Ford, R.D., Colom, L.V., and Bland, B.H. (1989). The classification of medial septum-diagonal band cells as σ -on or σ -off in relation to hippocampal EEG states. *Brain Research* *493*, 269-282.
- Foster, D.J., and Wilson, M.A. (2007). Hippocampal theta sequences. *Hippocampus* *17*, 1093-1099.
- Freund, T., and Antal, M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature* *336*, 170-173.
- Frotscher, M., and Léránth, C. (1985). Cholinergic innervation of the rat hippocampus as revealed by choline acetyltransferase immunocytochemistry: a combined light and electron microscopic study. *J. Comp. Neurol.* *239*, 237-246.
- Fuhrmann, F., Justus, D., Sosulina, L., Kaneko, H., Beutel, T., Friedrichs, D., Schoch, S., Schwarz, M.K., Fuhrmann, M., and Remy, S. (2015). Locomotion, Theta Oscillations, and the Speed-Related Firing of Hippocampal Neurons Are Controlled by a Medial Septal Glutamatergic Circuit. *Neuron* *86*, 1253-1264.
- Fyhn, M., Molden, S., Witter, M., Moser, E., and Moser, M.-B. (2004). Spatial representation in the entorhinal cortex. *Science* *305*, 1258-1264.
- Ganter, P., Szücs, P., Paulsen, O., and Somogyi, P. (2004). Properties of horizontal axo-axonic cells in stratum oriens of the hippocampal CA1 area of rats in vitro. *Hippocampus* *14*, 232-243.
- Gaykema, R., Luiten, P., Nyakas, C., and Traber, J. (1990). Cortical projection patterns of the medial septum-diagonal band complex. *J. Comp. Neurol.* *293*, 103-124.

Gaztelu, J.M., and Buno, W. (1982). Septo-hippocampal relationship during EEG theta rhythm. *Electroencephalogr Clin Neurophysiol* 54, 375-378.

Gielow, M.R., and Zaborszky, L. (2017). The input-output relationship of the cholinergic basal forebrain. *Cell Reports* 18, 1817-1830.

Givens, B., and Olton, D.S. (1994). Local modulation of basal forebrain: effects on working and reference memory. *J Neurosci* 14, 3578-3587.

Gogolak, G., Stumpf, C., Petsche, H., and Sterc, J. (1968). The firing pattern of septal neurons and the form of the hippocampal theta wave. *Brain Res* 7, 201-207.

Gonzalez-Sulser, A., Parthier, D., Candela, A., McClure, C., Pastoll, H., Garden, D., Sürmeli, G., and Nolan, M.F. (2014). GABAergic projections from the medial septum selectively inhibit interneurons in the medial entorhinal cortex. *J. Neurosci.* 34, 16739-16743.

Gray, E.G. (1959). Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *Journal of anatomy*, 420-433.

Hangya, B., Borhegyi, Z., Szilagy, N., Freund, T.F., and Varga, V. (2009). GABAergic neurons of the medial septum lead the hippocampal network during theta activity. *J. Neurosci.* 29, 8094-8102.

Hangya, B., Ranade, S.P., Lorenc, M., and Kepecs, A. (2015). Central Cholinergic Neurons Are Rapidly Recruited by Reinforcement Feedback. *Cell* 162, 1155-1168.

Hassani, O., Lee, M., Henny, P., and Jones, B. (2009). Discharge profiles of identified GABAergic in comparison to cholinergic and putative glutamatergic basal forebrain neurons across the sleep-wake cycle. *J. Neurosci.* 29, 11828-11840.

Hasselmo, M.E. (2005). What is the function of hippocampal theta rhythm? -Linking behavioral data to phasic properties of field potential and unit recording data. *Hippocampus* 15, 936-949.

Hasselmo, M.E., Bodelón, C., and Wyble, B.P. (2002). A proposed function for hippocampal theta rhythm: separate phases of encoding and retrieval enhance reversal of prior learning. *Neural computation* 14, 793-817.

Hinman, J.R., Brandon, M.P., Climer, J.R., Chapman, G.W., and Hasselmo, M.E. (2016). Multiple Running Speed Signals in Medial Entorhinal Cortex. *Neuron* 91, 666-679.

Huerta, P.T., and Lisman, J.E. (1993). Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. *Nature* 364, 723-725.

Huh, C.Y., Goutagny, R., and Williams, S. (2010). Glutamatergic neurons of the mouse medial septum and diagonal band of Broca synaptically drive hippocampal pyramidal cells: relevance for hippocampal theta rhythm. *J. Neurosci.* 30, 15951-15961.

Huxter, J.R., Senior, T.J., Allen, K., and Csicsvari, J. (2008). Theta phase specific codes for two-dimensional position, trajectory and heading in the hippocampus. *Nature neuroscience* 11, 587-594.

Jarosiewicz, B., McNaughton, B.L and Skaggs WE (2002). Hippocampal population activity during the small-amplitude irregular activity state in the rat. *J. Neurosci.* 15, 1373-1384

Jinno, S., Klausberger, T., Marton, L.F., Dalezios, Y., Roberts, J.D.B., Fuentealba, P., Bushong, E.A., Henze, D., Buzsáki, G., and Somogyi, P. (2007). Neuronal diversity in GABAergic long-range projections from the hippocampus. *J. Neurosci.* 27, 8790-8804.

- Jones, G.A., Norris, S.K., and Henderson, Z. (1999). Conduction velocities and membrane properties of different classes of rat septohippocampal neurons recorded in vitro. *J. Physiol.* 517, 867-877.
- Justus, D., Dalügge, D., Bothe, S., Fuhrmann, F., Hannes, C., Kaneko, H., Friedrichs, D., Sosulina, L., Schwarz, I., Elliott, D.A., *et al.* (2017). Glutamatergic synaptic integration of locomotion speed via septoentorhinal projections. *Nature neuroscience* 20, 16-19.
- Kadir, S.N., Goodman, D.F.M., and Harris, K.D. (2014). High-Dimensional Cluster Analysis with the Masked EM Algorithm. *Neural Computation* 26, 2379-2394.
- Kaifosh, P., Lovett-Barron, M., Turi, G.F., Reardon, T.R., and Losonczy, A. (2013). Septo-hippocampal GABAergic signaling across multiple modalities in awake mice. *Nat Neurosci* 16, 1182-1184.
- Kagi, U., Berchtold, M.W., and Heizmann, C.W. (1987). Ca²⁺-binding parvalbumin in rat testis. Characterization, localization, and expression during development. *J. Biol. Chem.* 262, 7314-7320.
- Kamondi, A., Acsady, L., Wang, X.-J., and Buzsaki, G. (1998). Theta oscillations in somata and dendrites of hippocampal pyramidal cells in vivo: Activity-dependent phase-precession of action potentials. *Hippocampus* 8, 244-261.
- Karson, M.A., Tang, A.H., Milner, T.A., and Alger, B.E. (2009). Synaptic cross talk between perisomatic-targeting interneuron classes expressing cholecystokinin and parvalbumin in hippocampus. *J Neurosci* 29, 4140-4454.
- Katona, L., Lapray, D., Viney, T.J., Oulhaj, A., Borhegyi, Z., Micklem, B.R., Klausberger, T., and Somogyi, P. (2014). Sleep and movement differentiates actions of two types of somatostatin-expressing GABAergic interneuron in rat hippocampus. *Neuron* 82, 872-886.
- Katona, L., Micklem, B., Borhegyi, Z., Swiejkowski, D.A., Valenti, O., Viney, T.J., Kotzadimitriou, D., Klausberger, T., and Somogyi, P. (2017). Behavior-dependent activity patterns of GABAergic long-range projecting neurons in the rat hippocampus. *Hippocampus* 27, 359-377.
- King, C., Recce, M., and O'Keefe, J. (1998). The rhythmicity of cells of the medial septum/diagonal band of Broca in the awake freely moving rat: relationships with behaviour and hippocampal theta. *Eur. J. Neurosci.* 10, 464-477.
- Kitamura, T., Pignatelli, M., Suh, J., Kohara, K., Yoshiki, A., Abe, K., and Tonegawa, S. (2014). Island cells control temporal association memory. *Science* 343, 896-901.
- Klausberger, T., Magill, P.J., Marton, L., Roberts, J.D.B., Cobden, P.M., Buzsáki, G., and Somogyi, P. (2003). Brain state- and cell type-specific firing of hippocampal interneurons in vivo. *Nature* 421, 844-848.
- Klausberger, T., and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* 321, 53-57.
- Koenig, J., Linder, A.N., Leutgeb, J.K., and Leutgeb, S. (2011). The spatial periodicity of grid cells is not sustained during reduced theta oscillations. *Science* 332, 592-595.
- Kohler, C., Chan-Palay, V., and Wu, J.-Y. (1984). Septal neurons containing glutamic acid decarboxylase immunoreactivity project to the hippocampal region in the rat brain. *Anat Embryol* 169, 41-44.

Kosaka, T. (1980). The axon initial segment as a synaptic site: ultrastructure and synaptology of the initial segment of the pyramidal cell in the rat hippocampus (CA3 region). *Journal of neurocytology* 9, 861-882.

Koutsoukos, E., Angelopoulos, E., Maillis, A., Papadimitriou, G.N., and Stefanis, C. (2013). Indication of increased phase coupling between theta and gamma EEG rhythms associated with the experience of auditory verbal hallucinations. *Neurosci Lett* 534, 242-245.

Kramis, R., Vanderwolf, C.H., and Bland, B.H. (1975). Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behavior and effects of atropine, diethyl ether, urethane, and pentobarbital. *Exp. Neurol.* 49, 58-85.

Kropff, E., Carmichael, J.E., Moser, M.B., and Moser, E.I. (2015). Speed cells in the medial entorhinal cortex. *Nature* 523, 419-424.

Kubota, Y., Shigematsu, N., Karube, F., Sekigawa, A., Kato, S., Yamaguchi, N., Hirai, Y., Morishima, M., and Kawaguchi, Y. (2011). Selective coexpression of multiple chemical markers defines discrete populations of neocortical GABAergic neurons. *Cereb Cortex* 21, 1803-1817.

Kunec, S., Hasselmo, M.E., and Kopell, N. (2005). Encoding and retrieval in the CA3 region of the hippocampus: a model of theta-phase separation. *J Neurophysiol* 94, 70-82.

Lapray, D., Lasztóczi, B., Lagler, M., Viney, T.J., Katona, L., Valenti, O., Hartwich, K., Borhegyi, Z., Somogyi, P., and Klausberger, T. (2012). Behavior-dependent specialization of identified hippocampal interneurons. *Nat. Neurosci.* 15, 1265-1271.

Lardi-Studler, B., Smolinsky, B., and Petitjean, C.M. (2007). Vertebrate-specific sequences in the gephyrin E-domain regulate cytosolic aggregation and postsynaptic clustering. *Journal of Cell Science* 120, 1371-1382.

Lasztóczi, B., and Klausberger, T. (2014). Layer-specific GABAergic control of distinct gamma oscillations in the CA1 hippocampus. *Neuron* 81, 1126-1139.

Lasztóczi, B., and Klausberger, T. (2016). Hippocampal Place Cells Couple to Three Different Gamma Oscillations during Place Field Traversal. *Neuron* 91, 34-40.

Lasztóczi, B., Tukker, J.J., Somogyi, P., and Klausberger, T. (2011). Terminal field and firing selectivity of cholecystokinin-expressing interneurons in the hippocampal CA3 area. *J. Neurosci.* 31, 18073-18093.

Lawson, V.H., and Bland, B.H. (1993). The role of the septohippocampal pathway in the regulation of hippocampal field activity and behavior: analysis by the intraseptal microinfusion of carbachol, atropine, and procaine. *Exp. Neurol.* 120, 132-144.

Leão, R.N., Mikulovic, S., Leão, K.E., Munguba, H., Gezelius, H., Enjin, A., Patra, K., Eriksson, A., Loew, L.M., Tort, A.B., *et al.* (2012). OLM interneurons differentially modulate CA3 and entorhinal inputs to hippocampal CA1 neurons. *Nature neuroscience* 15, 1524-1530.

Leao, R.N., Targino, Z.H., Colom, L.V., and Fisahn, A. (2015). Interconnection and synchronization of neuronal populations in the mouse medial septum/diagonal band of Broca. *J. Neurophysiol.* 113, 971-980.

Lee, A.M., Hoy, J.L., Bonci, A., Wilbrecht, L., Stryker, M.P., and Niell, C.M. (2014). Identification of a brainstem circuit regulating visual cortical state in parallel with locomotion. *Neuron* 83, 455-466.

Lee, B.H., Lamour, Y., and Bassant, M.H. (1991). Iontophoretic study of medial septal neurons in the unanesthetized rat. *Neurosci Lett* 128, 29-32.

- Lewis, P.R., and Shute, C.C. (1967). The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. *Brain* 90, 521-540.
- Li, S., Varga, V., Sik, A., and Kocsis, B. (2005). GABAergic control of the ascending input from the median raphe nucleus to the limbic system. *J. Neurophysiol.* 94, 2561 - 2574
- Linkenkaer-Hansen, K., Monto, S., Rytsala, H., Suominen, K., Isometsa, E., and Kahkonen, S. (2005). Breakdown of long-range temporal correlations in theta oscillations in patients with major depressive disorder. *J. Neurosci.* 25, 10131-10137.
- Lubenov, E.V., and Siapas, A.G. (2009). Hippocampal theta oscillations are travelling waves. *Nature* 459, 534-539.
- Luo, A.H., Tahsili-Fahadan, P., Wise, R.A., Lupica, C.R., and Aston-Jones, G. (2011). Linking context with reward: a functional circuit from hippocampal CA3 to ventral tegmental area. *Science* 333, 353-357.
- Magee, J.C., and Johnston, D. (1995). Synaptic activation of voltage-gated channels in the dendrites of hippocampal pyramidal neurons. *Science* 268, 301-304.
- Maurer, A.P., and McNaughton, B.L. (2007). Network and intrinsic cellular mechanisms underlying theta phase precession of hippocampal neurons. *Trends. Neurosci.* 30, 325 - 333
- McNaughton, N., Ruan, M., and Woodnorth, M.A. (2006). Restoring theta-like rhythmicity in rats restores initial learning in the Morris water maze. *Hippocampus* 16, 1102-1110.
- Melzer, S., Michael, M., Caputi, A., Eliava, M., Fuchs, E.C., Whittington, M.A., and Monyer, H. (2012). Long-range-projecting GABAergic neurons modulate inhibition in hippocampus and entorhinal cortex. *Science* 335, 1506-1510.
- Middleton, S.J., and McHugh, T.J. (2016). Silencing CA3 disrupts temporal coding in the CA1 ensemble. *Nature neuroscience* 19, 945-951.
- Miles, R., Traub, R.D., and Wong, R.K. (1988). Spread of synchronous firing in longitudinal slices from the CA3 region of the hippocampus. *J. Neurophysiol.* 60, 1481-1496.
- Mizumori, S.J., Perez, G.M., Alvarado, M.C., Barnes, C.A., and McNaughton, B.L. (1990). Reversible inactivation of the medial septum differentially affects two forms of learning in rats. *Brain Res* 528, 12-20.
- Mizumori, S.J., Ward, K.E., and Lavoie, A.M. (1992). Medial septal modulation of entorhinal single unit activity in anesthetized and freely moving rats. *Brain Res* 570, 188-197.
- Mizumori, S.J.Y., McNaughton, B.L., and Barnes, C.A. (1989). A comparison of supramammillary and medial septal influences on hippocampal field potentials and single-unit activity. *J. Neurophysiol.* 61, 15-31.
- Mizuseki, K., Royer, S., Diba, K., and Buzsáki, G. (2012). Activity dynamics and behavioral correlates of CA3 and CA1 hippocampal pyramidal neurons. *Hippocampus* 22, 1659-1680.
- Mizuseki, K., Sirota, A., Pastalkova, E., and Buzsáki, G. (2009). Theta oscillations provide temporal windows for local circuit computation in the entorhinal-hippocampal loop. *Neuron* 64, 267-280.
- Moser, M.B., and Moser, E.I. (1998). Functional differentiation in the hippocampus. *Hippocampus* 8, 608 - 619
- Nakamura, M., Sato, K., Fukaya, M., Araishi, K., Aiba, A., Kano, M., and Watanabe, M. (2004). Signaling complex formation of phospholipase C β 4 with metabotropic glutamate

receptor type 1a and 1,4,5-trisphosphate receptor at the perisynapse and endoplasmic reticulum in the mouse brain. *Eur. J. Neurosci.* 20, 2929-2944.

Neunuebel, J.P., and Knierim, J.J. (2014). CA3 retrieves coherent representations from degraded input: direct evidence for CA3 pattern completion and dentate gyrus pattern separation. *Neuron* 81, 416-427.

O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res* 34, 171-175.

O'Keefe, J. (1976). Place units in the hippocampus of the freely moving rat. *Exp. Neurol.* 51, 78-109.

O'Keefe, J. (1978). Large-amplitude irregular EEG activity (LIA). In *The Hippocampus as a cognitive map*, J. O'Keefe, and L. Nadel, eds. (Oxford University Press), pp. 150-153.

O'Keefe, J., and Nadel, L. (1978). *The hippocampus as a cognitive map* (Oxford: Clarendon).

O'Keefe, J., and Recce, M.L. (1993). Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus* 3, 317-330.

Orzel-Gryglewska, J., Matulewicz, P., and Jurkowlanec, E. (2015). Brainstem system of hippocampal theta induction: The role of the ventral tegmental area. *Synapse* 69, 553-575.

Pastalkova, E., Itskov, V., Amarasingham, A., and Buzsáki, G. (2008). Internally generated cell assembly sequences in the rat hippocampus. *Science* 321, 1322-1327.

Patel, J., Fujisawa, S., Berényi, A., Royer, S., and Buzsáki, G. (2012). Traveling theta waves along the entire septotemporal axis of the hippocampus. *Neuron* 75, 410-417.

Pernía-Andrade, A., and Jonas, P. (2014). Theta-Gamma-Modulated Synaptic Currents in Hippocampal Granule Cells In Vivo Define a Mechanism for Network Oscillations. *Neuron* 81, 1-13.

Petsche, H., Stumpf, C., and Gogolak, G. (1962). The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus I. The control of hippocampus arousal activity by the septum cells. *Electroencephalography and clinical neurophysiology* 14, 202-211.

Pinault, D. (1996). A novel single-cell staining procedure performed in vivo under electrophysiological control: morpho-functional features of juxtacellularly labeled thalamic cells and other central neurons with biocytin or neurobiotin. *J. Neurosci. Meth.* 65, 113-136.

Rajasethupathy, P., Sankaran, S., Marshel, J.H., Kim, C.K., Ferenczi, E., Lee, S.Y., Berndt, A., Ramakrishnan, C., Jaffe, A., Lo, M., *et al.* (2015). Projections from neocortex mediate top-down control of memory retrieval. *Nature* 526, 653-659.

Risold, P.Y., and Swanson, L.W. (1997). Connections of the rat lateral septal complex. *Brain research* 24, 115-195.

Robinson, J., Manseau, F., Ducharme, G., Amilhon, B., Vigneault, E., El Mestikawy, S., and Williams, S. (2016). Optogenetic Activation of Septal Glutamatergic Neurons Drive Hippocampal Theta Rhythms. *J. Neurosci.* 36, 3016-3023.

Roseberry, T.K., Lee, A.M., Lalive, A.L., Wilbrecht, L., Bonci, A., and Kreitzer, A.C. (2016). Cell-Type-Specific Control of Brainstem Locomotor Circuits by Basal Ganglia. *Cell* 164, 526-537.

Royer, S., Sirota, A., Patel, J., and Buzsáki, G. (2010). Distinct representations and theta dynamics in dorsal and ventral hippocampus. *J. Neurosci.* 30, 1777-1787.

- Saleem, A.B., Ayaz, A., Jeffery, K.J., Harris, K.D., and Carandini, M. (2013). Integration of visual motion and locomotion in mouse visual cortex. *Nature neuroscience* 16, 1864-1869.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., and Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature methods* 9, 676-682.
- Schomburg, E.W., Fernandez-Ruiz, A., Mizuseki, K., Berenyi, A., Anastassiou, C.A., Koch, C., and Buzsaki, G. (2014). Theta phase segregation of input-specific gamma patterns in entorhinal-hippocampal networks. *Neuron* 84, 470-485.
- Schwaller, B., Dick, J., Dhoot, G., Carroll, S., Vrbova, G., Nicotera, P., Pette, D., Wyss, A., Bluethmann, H., Hunziker, W., *et al.* (1999). Prolonged contraction-relaxation cycle of fast-twitch muscles in parvalbumin knockout mice. *American Journal of Physiology* 276, C395-C403.
- Shu, S.Y., Ju, G., and Fan, L.Z. (1988). The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. *Neurosci. Lett.* 85, 169-171.
- Siegle, J.H., and Wilson, M.A. (2014). Enhancement of encoding and retrieval functions through theta phase-specific manipulation of hippocampus. *Elife* 3, e03061
- Simon, A.P., Poindessous-Jazat, F., Dutar, P., Epelbaum, J., and Bassant, M.H. (2006). Firing properties of anatomically identified neurons in the medial septum of anesthetized and unanesthetized restrained rats. *J. Neurosci.* 26, 9038-9046.
- Singer, W. (1993). Synchronization of cortical activity and its putative role in information processing and learning. *Ann. Rev. Physiol.* 55, 349-374.
- Skaggs, W.E., McNaughton, B.L., Wilson, M.A., and Barnes, C.A. (1996). Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. *Hippocampus* 6, 149-172.
- Sławińska, U., and Kasicki, S. (1998). The frequency of rat's hippocampal theta rhythm is related to the speed of locomotion. *Brain Res* 796, 327-331.
- Solstad, T., Boccara, C.N., Kropff, E., Moser, M.-B.B., and Moser, E.I. (2008). Representation of geometric borders in the entorhinal cortex. *Science* 322, 1865-1868.
- Somogyi, P. (2010). Hippocampus - intrinsic organisation. In *Handbook of Brain Microcircuits*, G.M. Shepherd, and S. Grillner, eds. (Oxford: Oxford University Press), pp. 148-164.
- Somogyi, P., Katona, L., Klausberger, T., Lasztóczy, B., and Viney, T. (2014). Temporal redistribution of inhibition over neuronal subcellular domains underlies state-dependent rhythmic change of excitability in the hippocampus. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* 369, 20120518.
- Somogyi, P., Nunzi, M.G., Gorio, A., and Smith, A.D. (1983). A new type of specific interneuron in the monkey hippocampus forming synapses exclusively with the axon initial segments of pyramidal cells. *Brain Res* 25 137-142.
- Somogyi, P., and Takagi, H. (1982). A note on the use of picric acid-paraformaldehyde-glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry. *Neuroscience* 7, 1779-1783.
- Sotty, F., Danik, M., Manseau, F., Laplante, F., Quirion, R., and Williams, S. (2003). Distinct electrophysiological properties of glutamatergic, cholinergic and GABAergic rat

septohippocampal neurons: novel implications for hippocampal rhythmicity. *J. Physiol.* 551, 927-943.

Staudigl, T., and Hanslmayr, S. (2013). Theta oscillations at encoding mediate the context-dependent nature of human episodic memory. *Curr. Biol.* 23, 1101-1106.

Stewart, M., and Fox, S.E. (1990). Do septal neurons pace the hippocampal theta rhythm? *Trends. Neurosci.* 13, 163-168.

Stumpf, C., Petsche, H., and Gogolak, G. (1962). The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. *Electroencephalography and Clinical Neurophysiology* 14, 212-219.

Swanson, L.W., and Cowan, W.M. (1977). An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *J. Comp. Neurol.* 172, 49-84.

Swanson, L.W., and Cowan, W.M. (1979). The connections of the septal region in the rat. *J. Comp. Neurol.* 186, 621-655.

Sweeney, P., and Yang, Y. (2015). An excitatory ventral hippocampus to lateral septum circuit that suppresses feeding. *Nature communications* 6, 10188.

Tanaka, J., Nakagawa, S., Kushiya, E., Yamasaki, M., Fukaya, M., Iwanaga, T., Simon, M.I., Sakimura, K., Kano, M., and Watanabe, M. (2000). Gq protein α subunits G α_q and G α_{11} are localized at postsynaptic extra-junctional membrane of cerebellar Purkinje cells and hippocampal pyramidal cells. *Eur. J. Neurosci.* 12, 781-792.

Taube, J.S., Muller, R.U., and Ranck, J.B. (1990). Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. *J. Neurosci.* 10, 420-435.

Toth, K., Borhegyi, Z., and Freund, T.F. (1993). Postsynaptic targets of GABAergic hippocampal neurons in the medial septum diagonal band of Broca complex. *J. Neurosci.* 13, 3712-3724.

Tóth, K., Freund, T.F., and Miles, R. (1997). Disinhibition of rat hippocampal pyramidal cells by GABAergic afferents from the septum. *J. Physiol.* 500, 463-474.

Tsanov, M., Chah, E., Reilly, R., and O'Mara, S.M. (2014). Respiratory cycle entrainment of septal neurons mediates the fast coupling of sniffing rate and hippocampal theta rhythm. *Eur. J. Neurosci.* 39, 957-974.

Tukker, J.J., Lasztóczy, B., Katona, L., Roberts, J.D.B., Pissadaki, E.K., Dalezios, Y., Márton, L., Zhang, L., Klausberger, T., and Somogyi, P. (2013). Distinct dendritic arborisation and in vivo firing patterns of parvalbumin-expressing basket cells in the hippocampal area CA3. *J. Neurosci.* 33, 6809-6825.

Tukker, J.J., Tang, Q., Burgalossi, A., and Brecht, M. (2015). Head-directional tuning and theta modulation of anatomically identified neurons in the presubiculum. *J. Neurosci.* 35, 15391-15391.

Unal, G., Joshi, A., Viney, T.J., Kis, V., and Somogyi, P. (2015). Synaptic Targets of Medial Septal Projections in the Hippocampus and Extrahippocampal Cortices of the Mouse. *J. Neurosci.* 35, 15812-15826.

Vanderwolf, C.H. (1969). Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalogr Clin Neurophysiol* 26, 407-418.

- Varga, C., Golshani, P., and Soltesz, I. (2012). Frequency-invariant temporal ordering of interneuronal discharges during hippocampal oscillations in awake mice. *Proc Natl Acad Sci U S A* 109, E2726-E2734.
- Varga, C., Oijala, M., Lish, J., Szabo, G.G., Bezaire, M., Marchionni, I., Golshani, P., and Soltesz, I. (2014). Functional fission of parvalbumin interneuron classes during fast network events *eLife* 3, e04006.
- Varga, V., Losonczy, A., Zemelman, B.V., Borhegyi, Z., Nyiri, G., Domonkos, A., Hangya, B., Holderith, N., Magee, J.C., and Freund, T.F.F. (2009). Fast synaptic subcortical control of hippocampal circuits. *Science* 326, 449-453.
- Vertes, R., and Kocsis, B. (1997). Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. *Neuroscience* 81, 893-926.
- Viney, T., Lasztoczi, B., Katona, L., Crump, M., Tukker, J., Klausberger, T., and Somogyi, P. (2013). Network state-dependent inhibition of identified hippocampal CA3 axo-axonic cells in vivo. *Nature neuroscience* 16, 1802-1811.
- Wells, C.E., Amos, D.P., Jeewajee, A., Douchamps, V., Rodgers, J., O'Keefe, J., Burgess, N., and Lever, C. (2013). Novelty and anxiolytic drugs dissociate two components of hippocampal theta in behaving rats. *J. Neurosci.* 33, 8650-8667.
- Winson, J. (1972). Interspecies differences in the occurrence of theta. *Behavioral biology* 7, 479-487.-
- Winson, J. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science* 201, 160-163.
- Winter, S.S., Koppen, J.R., Ebert, T.B., and Wallace, D.G. (2013). Limbic system structures differentially contribute to exploratory trip organization of the rat. *Hippocampus* 23, 139-152.
- Witter, M.P. (2007). Intrinsic and extrinsic wiring of CA3: indications for connectional heterogeneity. *Learn. Mem.* 14, 705-713.
- Witter, M.P., Griffioen, A.W., Jorritsma-Byham, B., and Krijnen, J.L.M. (1988). Entorhinal projections to the hippocampal CA1 region in the rat: An underestimated pathway. *Neuroscience Letters* 85, 193-198.
- Wu, H., Williams, J., and Nathans, J. (2014). Complete morphologies of basal forebrain cholinergic neurons in the mouse. *eLife* 3.
- Xu, M., Chung, S., Zhang, S., Zhong, P., Ma, C., Chang, W.-C.C., Weissbourd, B., Sakai, N., Luo, L., Nishino, S., *et al.* (2015). Basal forebrain circuit for sleep-wake control. *Nature neuroscience.* 18, 1641-1647.
- Yartsev, M.M., Witter, M.P., and Ulanovsky, N. (2011). Grid cells without theta oscillations in the entorhinal cortex of bats. *Nature* 479, 103-107.
- Ylinen, A., Soltesz, I., Bragin, A., Penttonen, M., Sik, A., and Buzsaki, G. (1995). Intracellular correlates of hippocampal theta rhythm in identified pyramidal cells, granule cells, and basket cells. *Hippocampus* 5, 78-90.
- Zar, J.H. (1999). *Biostatistical Analysis*, 4th edn (New Jersey: Prentice-Hall Inc.).
- Zeisel, A., Muñoz-Manchado, A.B., Codeluppi, S., Lönnerberg, P., Manno, G., Juréus, A., Marques, S., Munguba, H., He, L., and Betsholtz, C. (2015). Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science* 347, 1138-1142.

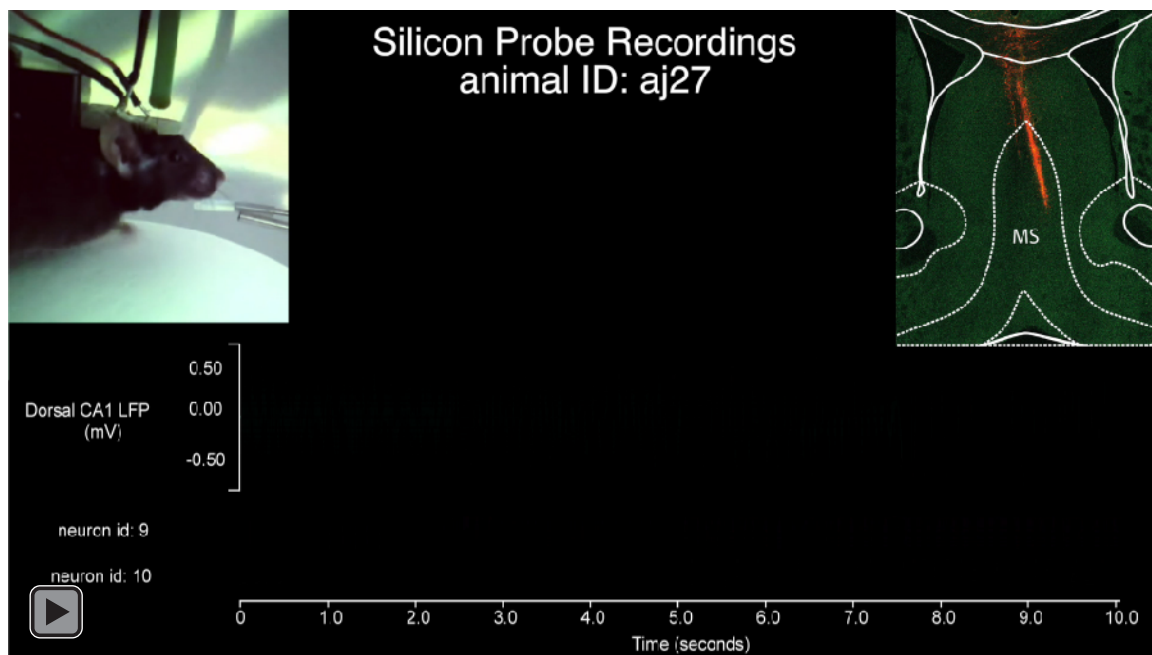
Videos



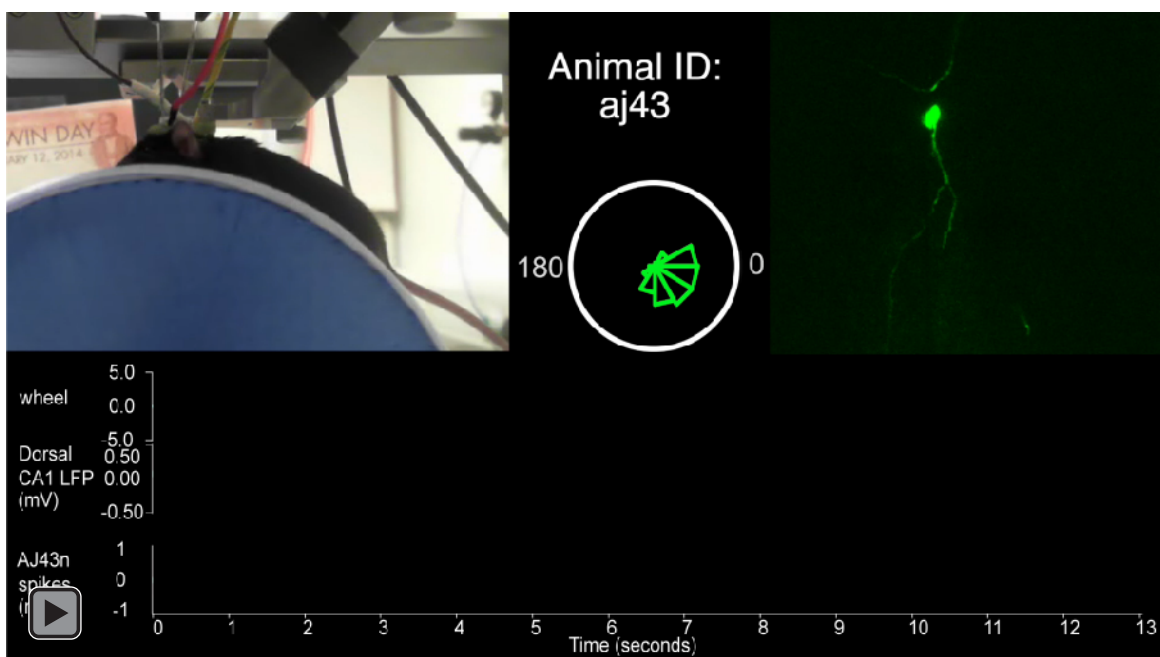
Video 2.1 Behaviours observed under head-fixation



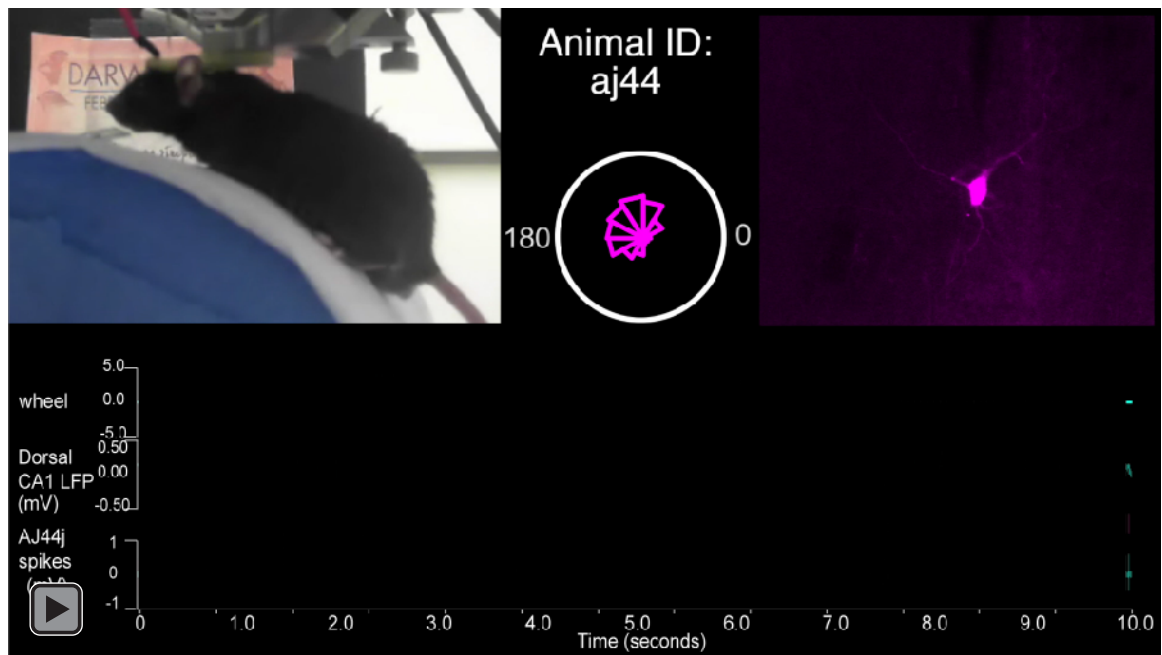
Video 3.1 Navigation in virtual reality



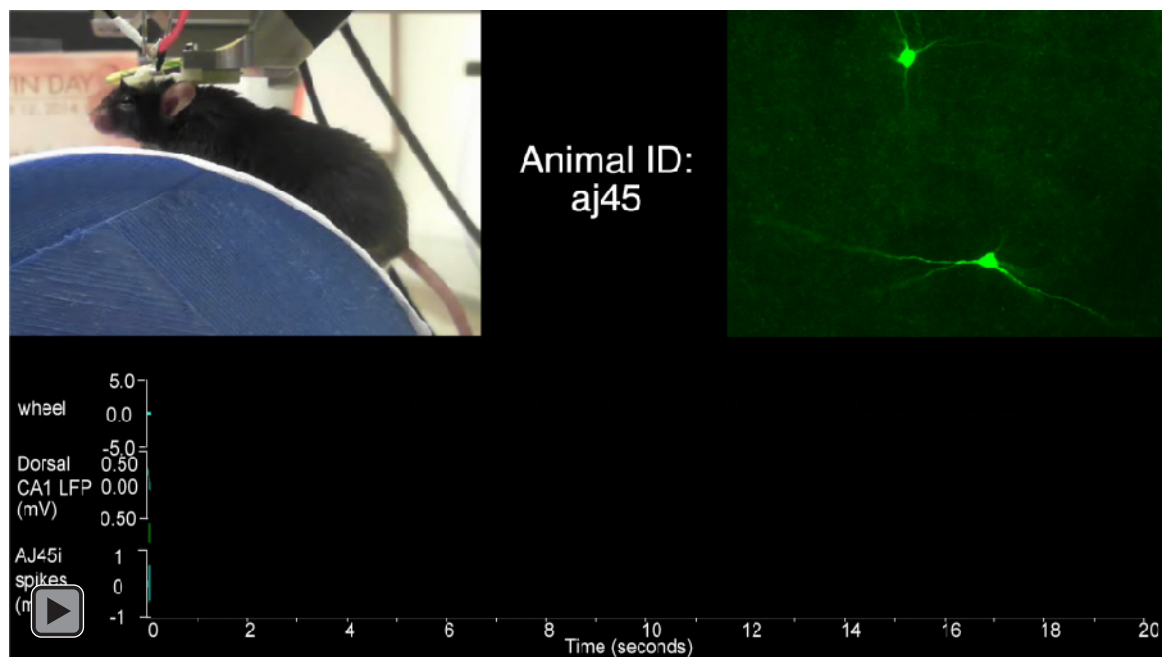
Video 3.2 *Teevra* and *Komal* neurons are identified by the ‘sound’ of their burst firing



Video 4.1 Activity patterns of *Teevra* cell, AJ43n during REST and RUN



Video 7.1 Activity patterns of *Komal* cell, AJ44j during REST and RUN



Video 7.2 Activity patterns of Group1 cell, AJ45i during REST and RUN