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Discussion
Introduction

In the previous chapter we assessed changes in CA1 neuronal and network activity caused by exposure to a single episode of 2 hour acute stress. We found that compared to awake immobility, during acute stress, CA1 neurons displayed suppressed firing while the amplitude and duration of CA1 ripples were enhanced. Short-term stress effects on the hippocampus are short-lived and subside within a few days (Shors et al., 1997) and are hypothesized to be adaptive. However, repeated activation of the body’s stress response by exposure to chronic stress has caused prolonged pathological changes in an animal’s physiology and cognition. Most such studies have assessed the effect of repeated stress on hippocampal structure and function only after the termination of chronic stress. Very little is known about the changes in hippocampal functionality during the course of chronic stress. Therefore, in this chapter we assessed the effect of repeated exposure to immobilization stress on CA1 neuronal and network activity and changes during exposure to 2 hours of immobilization stress. Before we go any further, let us briefly look at the effects of chronic stress on hippocampal morphology and synaptic plasticity that may potentially affect the activity and excitability of these neurons.

Chronic stress affects the anatomy and function of the hippocampus at multiple levels. Chronic stress causes the shortening and debranching of the principal neurons across hippocampal subregions including CA1 (Sousa et al., 2000; Christian et al., 2011), CA3 (Watanabe et al., 1992; Magariños and McEwen, 1995) and DG (Sousa et al., 2000). Dendritic spines, which are the postsynaptic sites of neurons, also undergo a decrease in number after exposure to chronic stress (Sandi et al., 2003; Pawlak et al., 2005; Stewart et al., 2005; but see Sunanda et al., 1995). Chronic stress causes impairment of long-term potentiation (LTP) in hippocampal slices (Kole et al., 2002; Pavlides et al., 2002; Alfarez et al., 2003; Radecki et al., 2005) and also in anesthetized animals (Gerges et al., 2001; Aleisa et al., 2006). Also, chronic stress facilitates hippocampal long term depression (Artola et al., 2006; Holderbach et al., 2007; Tran et al., 2011). Moreover, chronic stress also alters GABAergic inhibition in the hippocampus as chronically stressed animals display a 25-35% decrease in the number of perisomatic PV+ve inhibitory neurons across all regions of the hippocampus (Czeh et al., 2005; Hu et al., 2010). In agreement with these observations, in the Stress group, a greater fraction of the hippocampal neuronal population exhibits epileptic discharge in response to high frequency stimulation in anaesthetized animals (McEwen, 1999).

Alteration of dendritic structure (Narayanan and Chattarji, 2010), plasma Corticosterone (CORT) levels (Okuhara and Beck, 1998; Karst and Joëls, 2005), and GABAergic inhibition are three important co-factors that can affect neuronal firing and Local field potentials (LFPs). Previous reports suggest that stress may affect these co-factors in a temporal-specific manner.
For example, repeated exposure to the same stressor (homotypic stress) causes habituation of HPA-axis within a week from the start of the stress (Dhabhar et al., 1997; Garcia et al., 2000). Consequently, plasma CORT levels plateau off in less than a week from the initiation of the stress (Keim and Sigg, 1976; Kant et al., 1985; Pitman et al., 1988). Interestingly, dendritic atrophy is observed only in the later-half of the chronic stress regime (Magariños and McEwen, 1995). However, almost nothing is known about when in the chronic stress regime, the inhibitory neuronal loss is first observed.

Based on this evidence, it appears that different exposures to stress may differentially affect the neuronal and network activity in area CA1. While the habituation of the HPA-axis and plasma CORT levels during the first half of chronic stress suggests a decrease in neuronal activity (Melia et al., 1994). Dendritic atrophy has been hypothesized to cause enhanced excitability in hippocampal neurons (Narayanan and Chattarji, 2010). Similarly, impaired GABAergic inhibition in stressed animals (Hu et al., 2010) also suggests an increase in CA1 neuronal activity at the end of chronic stress. Therefore, to study the effect of repeated exposure to stress on hippocampal neurons, I monitored the pyramidal cell and ripple activity on Day-6 (D6, halfway time point of chronic stress), Day-10 (D10, on the last day of stress) and also on Day-11 (one day after the termination of chronic stress). I performed three types of comparisons:

i) Comparison between Rest and immobilization within a day

ii) Comparison during immobilization across experimental days (D1, D6 and D10)

iii) Comparison during immobilization between Control and Stress groups on experimental days one (D1), six (D6) and ten (D10)
Results

4.1 Effects of chronic stress on firing activity of CA1 pyramidal neurons during immobilization stress

To study the effects of chronic stress (2h/day for 10 consecutive days) on CA1 pyramidal neurons, we compared neuronal firing recorded during Rest (awake-immobility/sleep in a high walled sleep box) to that of the immobilization stress state. The two hour duration of immobilization stress was divided into four 30 minute periods: 0-30 min (First-30’), 30-60 min, 60-90 min and 90-120 (Last-30’). Since we intended to find out if 2h of stress will cause habituation of neuronal firing, the following section only focuses on data obtained during the First-30’ and the Last-30’ of immobilization stress (or time matched rest periods in the Control group). Since neuronal populations exhibited a non-normal distribution of activity during immobilization, the data below is presented as [median (inter-quartile range)]. Mean firing rate and Complex Spike Index (CSI) are the two readouts of neuronal firing that were assessed.

4.1.1 Comparison of neuronal activity during Rest, First-30’ and Last-30’ on Day-6:

![Figure 4.1 CA1 complex-spike cells display suppressed firing during exposure to immobilization stress on Day-6. (A) Schematic represents that on experimental day six, population activity of CA1 pyramidal cells is compared during Rest (awake immobility/sleep, white) and later when mice experience immobilization stress. The 2h immobilization period is divided into four 30 min episodes, i.e. 0-30 min (First-30’), 30-60 min, 60-90 min, and 90-120 min (Last-30’). Here we only analysed the First-30’ (light gray) and Last-30’ (dark gray) of the immobilization stress. (B) CA1 neurons exhibit significantly lower mean firing (Hz) during First-30’ (p<0.05) and Last-30’ (p<0.01) as compared to the Rest epoch. (C) Complex spike index (CSI) that provides a readout of neuronal bursting activity exhibits no significant change during either First-30’ (p>0.05) or Last-30’ (p>0.05) of stress. Bars represent inter-quartile-range (25th-75th percentile), and the horizontal lines within the bars represent median values. Small squares represent mean values. KW-ANOVA followed by a pair wise comparison using MWU post hoc test.*p<0.05, **p<0.01.](image)
4.1.1 Chronic stress reduced firing during First-30’ and Last-30’: On experimental day six (D6), CA1 neuronal firing was compared during Rest (white, n=71 cells), First-30’ (light gray, n=34 cells) and Last-30’ (dark gray, n=27 cells) epochs (Fig 4.1 A). Repeated stress for five days did not block immobilization-induced suppression of neuronal firing. Similar to D1, mean firing rate (Hz) displayed a difference during the above three states on D6 \( \chi^2 (2) = 12.61613, p<0.01 \). Relative to Rest \([0.61 (0.92)]\), mean firing exhibited a decrease during First-30’ \([0.38 (0.41), p<0.05]\), and Last-30’ \([0.29 (0.27), p<0.01]\). No difference in mean firing was observed between First-30’ and Last-30’ \( p>0.05 \) (Fig 4.1 B). Complex Spike index (CSI) displayed similar values during Rest \([14.30 (11.27)]\), First-30’ \([14.68 (11.55)]\) and Last-30’ \([15.85 (12.42)]\) and no significant difference was observed during stress \( F_{2, 124} = 1.17023, p>0.05 \) (Fig 4.1C).

4.1.1.2 Control group displayed similar firing across Rest, First-30’ and Last-30’: Another group of animals that never experienced stress served as the Control group. To provide time matched data, neuronal firing in Control group is compared during Rest (n=70 cells) and during another 2h of rest period. This two hour duration of rest was divided into four 30 minute periods: 0-30 min (First-30’), 30-60 min, 60-90 min and 90-120 (Last-30’). Here we only considered First-30’ (n=25cells) and Last-30’ (n=26 cells) (Fig 4.2 A).

![Figure 4.2](image.png)

*Figure 4.2* Control group exhibits similar firing and bursting activity across Rest states on Day-6 (A) Schematic represents that in the control group, population activity of CA1 pyramidal cells is compared during multiple rest states, i.e. Rest (awake immobility/rest), First-30’ (awake immobility/rest, time-matched to stress First-30’) and Last-30’; (awake immobility/rest activity, time-matched to stress Last-30’). (B) CA1 neurons exhibit similar mean firing (Hz) during Rest, First-30’ and Last-30’. (C) Complex Spike Index also exhibits similar values during Rest, First-30’ and Last-30’. Bars represent inter-quartile range (25th-75th percentile), and horizontal line within bars represents median values. Small squares represent mean value.
As expected, Control group exhibited similar mean firing (Hz) during Rest [0.88 (1.15)], First-30’ [0.76 (0.87)] and Last-30’ [0.72 (1.23)] and hence no significant difference was observed among them [F_{2, 117} = 0.297, p>0.05] (Fig 4.2 B). Similarly, neuronal bursting exhibited no difference across three rest states [F_{2, 117} = 2.85648, p>0.05]. As compared to Rest [18.43 (12.88)], CSI value displayed a slight, but non-significant, increase during First-30’ [22.78 (17.00)] which subsided by Last-30’ [17.84 (22.69)] (Fig. 4.2 C).

4.1.2 Comparison of neuronal activity of cells that are active during both First-30’ and Last-30’ on Day-6: In the previous sections we compared firing of CA1 pyramidal cells during Rest, and stress (First-30’ and Last-30’). While this analysis provided important information about how a neuronal ensemble gets affected by stress, it is likely that some cells go silent while others exhibit enhanced activity during one of these three periods. This may potentially mask some of the stress effects. Therefore, next we compared activity of only those cells that were active during First-30’ and Last-30.

4.1.2.1 Majority of Stress group cells displayed a decrease in mean firing: In the Stress group, 14 cells were active during First-30’ and Last-30’. The majority of cells (10 out of 14) displayed a decrease in mean firing. However, no significant difference was observed in average mean firing between First-30’ and Last30’ [First-30’: 0.54 ± 0.15 Vs Last-30’: 0.55 ± 0.16, p>0.05] (Fig 4.3 A). 7 out of 14 cells exhibited an increase while remaining 7 cells displayed a decrease in CSI value during Last-30’. Furthermore, no significant difference (p>0.05) was observed in average CSI value between First-30’ (15.67 ± 1.95) and Last-30’ (17.26 ± 2.49) (Fig 4.3 A).

4.1.2.2 Majority of Control group cells displayed a decrease in neuronal bursting: In the Control group, 15 cells were active during First-30’ and Last-30’. Interestingly, unlike stress group, half of the population (8 cells) displayed an increase in mean firing while remaining 7 cells showed a decrease. In agreement, average mean firing exhibited almost identical values during First-30’ (1.17 ± 0.27) and Last-30’ (1.22 ± 0.30) (Fig 4.3 B). Unlike Stress group, a majority of CA1 cells (10 out 15 cells) in Control group displayed a decrease in CSI value. However, average CSI value did not show a significant difference (p>0.05) between First-30’ (27.08 ± 2.85) and Last-30’ (25.63 ± 3.78) (Fig 4.3 B).

4.1.3 Comparison of neuronal activity during First-30’ and Last-30’ on Day-10: As explained in Chapter-2 (materials and methods), we did not design our experiment to record Rest data from Stress group on D10. Therefore, next we compared the firing activity of CA1 pyramidal cells during First-30’ and Last-30’ on D10.
4.1.3.1 Stress group displayed enhanced neuronal bursting during Last-30’ on Day-10:

Next we compared mean firing and neuronal bursting activity in Stress group during First-30’ (n=14 cells) and Last-30’ (n=16 cells) on D10. In Stress group, mean firing (Hz) exhibited very similar values during First-30’ and Last-30’ and hence no significant difference was observed (First-30’: 0.63 ± 0.24, Vs Last-30’: 0.50 ± 0.11, p>0.05). 5 out of 7 cells, which were active during both phases of stress, exhibited an increase in mean firing (Fig 4.4 C). Interestingly, neuronal bursting displayed a significant enhancement as CSI showed greater value during Last-30’ (First-30’: 14.27 ± 1.69, Vs Last-30’: 21.56 ± 2.75, p<0.05). In support of this observation, 6 out of 7 cells that were active during both phases, exhibited a greater CSI value during Last-30’ (Fig 4.4 C).
4.1.3.2 Control group displayed lower mean firing during Last-30’ on Day-10: Next we analysed the neuronal activity from Control group during First-30’ (n=25 cells) and Last-30’ (n=29 cells). Mean firing exhibited a significant decrease during late phase of immobilization (First-30’: 0.87 ± 0.17, Vs Last-30’: 0.48 ± 0.06, p<0.05). Interestingly, half of the cell population (9 out of 17 cells) that was active during First-30’ and Last-30’, exhibited an increase while the remaining 8 cells displayed a decrease in mean firing during Last-30’ (Fig 4.5 B). Similarly, First-30’ and Last-30’ showed almost identical CSI values (First-30’: 16.31 ± 1.76, Vs Last-30’: 16.94 ± 1.71, p>0.05). While 7 cells displayed an increase, 10 cells displayed a decrease in CSI value during Last-30’ (Fig 4.5 C).
4.1.4 Comparison of neuronal activity during First-30’ on Day-1, Day-6 and Day-10:

Another way to analyse the effect of chronic stress on CA1 pyramidal cells is to compare the population activity after various exposures to a stressor (across day comparison). Therefore, next we compared neuronal activity across D1, D6 and D10 (Fig 4.6). It should be noted that since the number of cells recorded on D10 were very few in stress group, we intended to use this data only to get trends of stress effects on D10.

4.1.4.1 Stress group exhibited a decrease in neuronal bursting during First-30’ on Day-6 and Day-10:

We first compared neuronal firing during First-30’ of immobilization stress on D1 (n=30 cells), D6 (n=34 cells) and D10 (n=14 cells) (Fig 4.6 A). Repeated exposure to stress,
affected neuronal bursting \[F_{2, 75} = 4.85, p<0.05\]. As compared to D1 [23.81 (14.76)], CSI value was reduced on D6 [14.70 (11.55), \(p<0.05\)] and also on D10 [12.62 (10.28), \(p<0.05\)]. However, CSI did not differ between D6 and D10 (\(p>0.05\)) (4.6 B top). Chronic stress did not affect average firing (Hz) during First-30’ across days \[\chi^2 (2) = 1.08, p>0.05\]; 4.6 B bottom.

4.1.4.2 Control group also exhibited suppressed neuronal bursting but only on Day-10:
Next we analysed neuronal firing during First-30’ in Control group on D1 (n=27 cells), D6 (n=25 cells) and D10 (n=25 cells). Mean firing (Hz) did not differ across days \[F_{2, 74} = 2.07, p>0.05\]. In comparison to D1 [0.47 (0.63)], mean firing was slightly enhanced on D6 [0.76 (0.87)] but came down on D10 [0.42 (1.17)] (Fig 4.6 D top). Interestingly, neuronal bursting
showed a significant difference across days \([F_{2, 74} = 5.30, p<0.05]\). CSI did not differ between D1 and D6 \([D1: 23.83 (17.11) Vs D6: 22.78 (17.0), p>0.05]\). However, CSI value on D10 displayed a significantly lower value \([15.17 (9.91)]\), relative to D1 \((p<0.05)\) and D6 \((p<0.01)\) (Fig 4.6 D bottom).

### 4.1.5 Comparison of neuronal firing between Control and Stress groups on Day-1, Day-6 and Day-10 (Table 4.1)

<table>
<thead>
<tr>
<th>Day</th>
<th>Mean firing (Hz)</th>
<th>Complex spike index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Stress</td>
</tr>
<tr>
<td>D-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-30’</td>
<td>0.47 (0.63)</td>
<td>0.32 (0.57)</td>
</tr>
<tr>
<td>Last-30’</td>
<td>0.40 (0.70)</td>
<td>0.32 (0.45)</td>
</tr>
<tr>
<td>D-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-30’</td>
<td>0.76 (0.87)</td>
<td>0.38 (0.41)</td>
</tr>
<tr>
<td>Last-30’</td>
<td>0.72 (1.23)</td>
<td>0.29 (0.28) (p&lt;0.05)</td>
</tr>
<tr>
<td>D-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-30’</td>
<td>0.42 (1.17)</td>
<td>0.20 (0.56)</td>
</tr>
<tr>
<td>Last-30’</td>
<td>0.37 (0.34)</td>
<td>0.29 (0.88)</td>
</tr>
</tbody>
</table>

Table 4.1: Neuronal activity comparison between Control and Stress group during either First-30’ or Last-30’ on experimental days one (D1), six (D6) and ten (D10). Stress group exhibits a trend of lower mean firing (Hz) than that of Stress group on all the days. However the significant difference was observed only on D6. Neuronal bursting displayed lower value in Stress group on D6 and D10. However, CSI value displayed a significantly lower value in Stress group on D6. Data is presented as median(inter-quartile-range). Unpaired t-test or MWU test.

### 4.2 Effect of chronic stress on CA1 ripple activity during immobilization stress

In the previous chapter, we observed that exposure to immobilization stress caused an increase in ripple amplitude and duration. Since chronic stress causes structural and synaptic plasticity changes in both excitatory and inhibitory neurons, we intended to study the effect of stress on ripple activity which is generated by the interplay of network excitation and inhibition. In this section we analysed the effect of chronic stress on ripple activity during exposure to stress on D6 and D10. Data is presented as Mean \(\pm\) SEM of these values.
4.2.1 Comparison of ripple activity during Rest, First-30’ and Last-30’ on Day-6: We compared CA1 ripple activity across Rest, First-30’ and Last-30’ on D6 (Fig 4.7 A).

Table 4.7 Chronic stress affects ripple characteristics during subsequent immobilization on Day-6. (A) Schematic shows that on experimental day six (D6), CA1 ripple activity is compared during Rest (white), First-30’ (light gray) and Last-30’ (dark gray). The data is presented as percentage of the Rest value. (B) Stress group exhibits slightly bigger ripple amplitude but it was an insignificant effect during First-30’ (p>0.05) and Last-30’ (p>0.05). (C) Similarly, ripple length exhibits a trend toward greater value during stress. As compared to Rest, ripple length displays a trend of greater value during First-30’ (p=0.05) and Last-30’ (p=0.052). (D) Inter-ripple-interval (IRI) exhibits increasing trend during Last-30’ (p=0.05).

4.2.1.1 Chronic stress affected immobilization-induced increase in ripple amplitude on Day-6: Similar to D1, ripple amplitude (mV) in Stress group exhibited an increasing trend during First-30’ (161.9 ± 34.4) and Last-30’ (145.9 ± 31.7) on D6. However, because of the greater variability, no significant differences in ripple amplitude were observed between Rest, First-30’ and Last-30’ [F1.0, 8.91 = 2.8, p>0.05; Fig 4.7 B]. The Control group exhibited similar ripple amplitude across all rest states on D6 [e.g. Rest: 100 ± 0.0, First-30’: 103.3 ± 14.2 and Last-30’: 94.7 ± 14.4], and no significant differences were observed among them [F1.0, 10.9 = 0.28, p>0.05; Fig 4.8 B].

4.2.1.2 Chronic stress also affected immobilization-induced increase in ripple length on Day-6: Similar to D1, Stress group displayed significant differences in ripple duration (ms) between Rest, First-30’ and Last-30’ in the Stress group [F2.3 = 5.06, p<0.05]. Exposure to chronic stress caused a slight, but insignificant, increase in ripple length during First-30’ (113.8 ± 5.13; p=0.07) and Last-30’ (114.6 ± 6.7; p=0.052), as compared to Rest (100.0). Ripple length showed no difference between First-30’ and Last-30’ (p>0.05; Fig 4.7 C). As expected, Control
group exhibited similar ripple length during all rest states [e.g. Rest: 100 ± 0.0, First-30’: 103.3 ± 14.2 and Last-30’: 94.7 ± 14.4] and hence no significant difference across rest state was observed \([F_{2,5.04} = 0.04, p>0.05; \text{Fig 4.8 C}]\).

**4.2.1.3 Chronic stress did not affect inter-ripple-interval (IRI) on Day-6:** Even though Stress group displayed a trend of greater IRI (s) during Last-30’ (160.4± 26.8), because of greater variability, no significant difference was observed across Rest, First-30’ and Last-30’ \([F_{2,7.83} = 5.46, p>0.05; \text{Fig 4.7 D}]\). Similarly, Control group exhibited similar IRI values during all rest states [e.g. Rest: 100 ± 0.0, First-30’: 97.6 ± 10.0 and Last-30’: 105.4 ± 13.1], and no significant differences across rest states were observed \([F_{2,2.24} = 0.30, p>0.05; \text{Fig 4.8 B}]\).

**4.2.2 Comparison of CA1 ripple activity during First-30’ or Last-30’ on Day-1, Day-6 and Day-10:** Next we compared the ripple activity during First-30’ or Last-30’ epochs during either immobilization or during Rest (for Control group) across D1, D6 and D10. Chronic stress did not have a stronger effect on ripple activity during immobilization (either-First-30’ or Last-30’) on D1, D6 and D10 except that Stress group displayed a slight but non-significant increase in IRI value on D10 (See table 4.2).
4.2.3 Comparison of CA1 ripple activity between Control and Stress group on Day-1, Day-6 or Day-10

4.2.3.1 Stress group exhibited greater ripple length on Day-1: Stress group showed slightly bigger ripple amplitude (mV) than Control group on D1. However, this was not significant either during First-30’ (Stress: 0.62 ± 0.08 Vs Control: 0.50 ± 0.10, p>0.05) or Last-30’ (Stress: 0.56 ± 0.08 Vs Control: 0.45 ± 0.08, p>0.05; Fig 4.9 B). Immobilization stress caused a significant increase in ripple duration (ms) as Stress group exhibited significantly greater ripple length than Control group during First-30’ (Stress: 77.95 ± 4.42 Vs Control: 62.24 ± 2.41, p<0.05), as well as Last-30’ (Stress: 77.80 ± 4.80 Vs Control: 60.51 ± 1.76, p<0.05 ; Fig 4.9 C). While ripple occurrence displayed a slight decrease in Stress group, it was not a significant effect. Inter-ripple-interval (IRI) exhibited an insignificant increase in Stress group during First-30’ (Stress: 2.39 ± 0.42 Vs Control: 1.68 ± 0.11, p>0.05) or Last-30’ (Stress: 2.62 ± 0.32 Vs Control: 1.76 ± 0.10, p>0.05; Fig 4.9 D).
4.2.3.2 Stress group exhibited no difference in ripple activity on Day-6: Though, on D6 Stress group displayed slightly bigger ripple amplitude (mV) than Control group, no significant differences were observed between Stress and Control groups during either First-30’ (Stress: 0.65 ± 0.10 Vs Control: 0.44 ± 0.08, p>0.05) or Last-30’ (Stress: 0.59 ± 0.09 Vs Control: 0.40 ± 0.07, p>0.05) (Fig 4.10 B). On D6, ripple length (ms) in Stress group exhibited a slightly greater value though no significant differences were observed between Control and Stress group during either First-30’ (Stress: 71.27 ± 3.35 Vs Control: 60.58 ± 4.39, p>0.05), or Last-30’ (Stress: 71.96 ± 4.85 Vs Control: 59.88 ± 4.46, p>0.05; Fig 4.10 C). Similarly, while inter-ripple-interval (s) showed greater value in Stress group, no significant differences were observed between groups during either First-30’ (Stress: 1.98 ± 0.42 Vs Control: 1.76 ± 0.08, p>0.05) or Last-30’ (Stress: 2.97 ± 0.58 Vs Control: 1.90 ± 0.17, p>0.05; Fig 4.10 D).
4.2.3.3 Stress group exhibited greater ripple length and inter-ripple-interval on Day-10:

On D10, ripples exhibited similar amplitude (mV) between the Control and the Stress group during First-30’ (Stress: 0.50 ± 0.05 Vs Control: 0.50 ± 0.11, p>0.05) and Last-30’ (Stress: 0.46 ± 0.03 Vs Control: 0.51 ± 0.06, p>0.05; Fig 4.11 B). Interestingly, the Stress group exhibited significantly longer ripple length (ms) as compared to the Control group during First-30’ (Stress: 73.19 ± 5.20 Vs Control: 57.74 ± 2.63, p<0.05) but not during Last-30’ (Stress: 72.09 ± 6.03 Vs Control: 62.69 ± 1.32, p>0.05; Fig 4.11 C). Inter-ripple-interval (s) exhibited no difference between Control and Stress groups during First-30’ (Stress: 3.37 ± 1.16 Vs Control: 2.86 ± 0.72, p>0.05) but displayed significantly greater IRI value during last-30’ (Stress: 3.61 ± 0.62 Vs Control: 1.71 ± 0.67, p>0.05; Fig 4.11 D).
Discussion

Chronic stress affects structure and function of the hippocampus (see reviews by McEwen, 1999; Lupien and Lepage, 2001; Kim and Diamond, 2002; McEwen and Chattarji, 2006; Joëls et al., 2007). The majority of studies have examined the effects of chronic stress on hippocampal neurons after the termination of stress. However, very is known about the progression of changes in neuronal and network activity during the course of chronic stress. Therefore, in the current chapter, I asked if repeated exposure to stress will affect the ability of CA1 complex-spike cells to lower their firing rate during exposure to immobilization stress as was observed during first exposure to immobilization (Fig 3.1 in Chapter-3). Moreover, should chronic stress also affect the immobilization-induced increase in CA1 ripple amplitude and duration as seen in Fig 3.4 (Chapter-3)? Therefore, to study the effect of chronic stress (immobilization 2h/day for 10 consecutive days) on CA1 neuronal and network activity, pyramidal cell and ripple activity was recorded on D6 (after 5 days of stress) and on D10 (on the last day of chronic stress).

**Chronic stress effects on CA1 complex spike cell firing:** Similar to D1 results, CA1 neurons still exhibited suppressed mean firing during First-30’ and Last-30’, relative to Rest (Fig 4.1). This observation suggested that repeated exposure to stress does not cause a habituation effect *per se*. However, the decrease in neuronal firing during First-30’ dropped from 2.62 fold...
Chronic stress effects on pyramidal cell firing and ripple activity in area CA1

(p<0.001) on D1 to 1.76 fold (p<0.05) on D6. Similar to D1, neuronal bursting still did not show a decrease during immobilization, as compared to Rest on D6. Next, we compared the firing of CA1 neurons during immobilization on D1, D6, and D10. While chronic stress did not affect mean firing during immobilization across days, neuronal bursting displayed a decrease, as CSI showed a significantly lower value during First-30’ on D6 (p<0.05) and also on D10 (p<0.05) (Fig 4.6). Interestingly, Control group also displayed a significantly lower CSI value during First-30’ on D10, but not on D6. Since, in Control group, peak firing also showed a significant enhancement on D10, it appears that Control animals were more active during First-30’ on D10 as compared to either D1 or D6 (Fig 4.6). Another type of analysis, that involved comparison of neuronal firing between Control and Stress groups during First-30’ or Last-30’ showed lower mean firing and neuronal bursting (CSI) on D6. Taken together, the above evidence suggests that in comparison to Rest, the exposure to immobilization stress causes a reduction in mean firing and doe not affect neuronal bursting (Fig 3.1). However, exposure to chronic stress impedes/suppresses the ability of CA1 neurons to undergo neuronal bursting during immobilization, as seen on D6 and D10 (Fig 4.6).

Chronic stress effects on CA1 ripple activity: In the last chapter, we found an increase in amplitude and duration of CA1 ripples during first exposure to immobilization. Similar to D1 results, even on D6, we found a trend of greater ripple amplitude and length during immobilization on D6. However, unlike D1, this increase in ripple properties was not a significant effect (Fig 4.7). This observation suggests that chronic stress did affect immobilization-induced increase in ripple activity on D6. As mentioned earlier, our experiments were not designed to record Rest activity on D10 (though we recorded ripple activity during immobilization on D10) the above comparison could not be made on D10. Next, we made a comparison of ripple characteristics during immobilization across D1, D6 and D10. A trend of reduced ripple amplitude and fewer occurrences of ripples were observed with repeated exposure to stress but it was not a significant effect (Table-4.2). This result again points to the fact that chronic stress does not strongly affect ripple characteristics during immobilization. Next, we compared the ripple activity between Control and Stress groups. This comparison indicated that CA1 ripples tend to be broader as ripple length was greater in Stress group across all recording days (Figs 4.9 and 4.11). Moreover, Stress group also displayed enhanced inter-ripple-interval (IRI) on D10 again suggesting fewer occurrence of ripples. Taken together it appears that exposure to immobilization stress causes an increase in ripple length and amplitude as observed on D1 (Fig 3.4). However, repeated stress weakly affects CA1 neuronal network’s ability to increase amplitude and duration of ripple during immobilization on D6 and D10.
But what does chronic stress-induced broadening of ripples (increased ripple length) and reduced frequency of ripple occurrence mean? As discussed earlier, the co-activity of hippocampal neurons shows an enhancement during ripples. Thus enhanced ripple length suggests an increase in neuronal co-activity during immobilization on D10. However, greater IRI points to fewer incidences of CA1 neuronal co-firing, especially during Last-30’ on D10 (Fig 4.11 C). Earlier reports of enhanced magnitude and duration of ripples points to memory consolidation after associative learning (Eschenko et al., 2008). Therefore, increased ripple amplitude and length during immobilization (as compared to Rest) on D1 and D6 may facilitate contextual representation of the linear tracks that animals visited prior to stress (linear-track experiments are discussed in chapter-5).