The discussion, conclusions and future directions

Overview

Discussion
8.1 Suppressed CA1 pyramidal neuronal firing during the exposure to the immobilization stress- \textit{in vivo} recordings
8.2 Enhanced CA1 ripple activity during the exposure to immobilization stress- \textit{in vivo} recordings
8.3 CA1 place cells from the Stress group fail to modulate their firing or field size – \textit{in-vivo} recordings
8.4 Chronic stress causes enhancement of contextual fear learning, especially if exposure to the context was short during conditioning-behavioural experiments
8.5 Chronic stress affects time interval coding in one-trial contextual fear conditioning- behavioural experiments
8.6 Stress facilitates acquisition of amygdala-mediated auditory fear conditioning
8.7 Similarities and contrast between chronic stress and aging (table 8.1) – amygdala dominance decides the freezing during contextual fear conditioning

Conclusions and future directions
Overview

In this thesis, I have addressed the impact of chronic stress on spatial encoding by exposing rodents to multiple spatial contexts. This was achieved by assessing various properties (firing rate, field size etc) of CA1 place cells that fire repeatedly and reliably in a location-specific manner in its surroundings. The comparison of CA1 place cell activity indicated that stressed mice were unable to modulate firing rate or place field size between familiar and novel tracks (Chapter-5). An important question that remains unanswered is how the hippocampal neuronal network responds during the exposure to a stressor. The very few studies that have addressed this question by employing the stress hormone corticosterone (CORT), as a proxy for stress, have provided mixed results (Pfaff et al., 1971; Barak et al., 1977; Yamato et al., 2002). Thus CA1 neuronal and network activity was assessed during the exposure to immobilization stress. CA1 neurons displayed suppressed neuronal firing throughout the 2hrs of immobilization stress (Chapter-3 and 4). Furthermore, an analysis was also made to assess the effect of sharp-wave ripples, an ultrafast oscillatory state of the hippocampal network, which has been associated with memory consolidation. CA1 ripples displayed larger amplitude and length during immobilization (Chapter-3 and4).

Interestingly, chronic stress impairs spatial memory while various other associative learning (Pavlovian conditioning) paradigms display an enhancement. The second half of this thesis tested these paradigms in control and stressed animals and found that chronic immobilization stress causes enhancement of contextual fear conditioning (CFC; Chapter-6) and auditory fear conditioning (AFC Chapter-7). Furthermore, the encoding of time intervals was also affected in stressed rats (Chapter-6). In addition, stressed animals also displayed enhanced anxiety-like behaviour (Chapter-7).

Based on the above results obtained from my experiments, the previous work from our laboratory and available literature (especially on aging); in this thesis it is reasoned that amygdala hyperactivity plays a dominant role in spatial and contextual encoding. This line of reasoning thus suggests that enhanced contextual fear may not be specific to the conditioning context i.e. animals may display similarly high freezing in the conditioning context as well as in another similar but not identical spatial context. This thesis thus suggests that associative learning, under aversive conditions, should be considered as a paradigm that is mediated by the hippocampus-amygdala circuitry and not merely a hippocampus-mediated behaviour. This context generalization, perhaps facilitated by the amygdala hyperactivity, can thus give rise to anxiety-like behaviour observed in stressed patients and animal models of stress.
# Chronic stress effects

**Network (in vivo) - Hippocampus**

<table>
<thead>
<tr>
<th>Chapter 3, 4</th>
<th></th>
<th>Chapter 5</th>
<th></th>
<th>Chapter 6, 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1 firing-immobilization</td>
<td>↓</td>
<td>CA1 Rate remapping</td>
<td>×</td>
<td>Generalized fear (anxiety)</td>
</tr>
<tr>
<td>CA1 Ripple activity</td>
<td>↑</td>
<td>CA1 firing - rest</td>
<td></td>
<td>Cued fear (AFC)</td>
</tr>
</tbody>
</table>

**Behaviour**

<table>
<thead>
<tr>
<th>Chapter 6, 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trace conditioning</td>
</tr>
<tr>
<td>Interval encoding</td>
</tr>
<tr>
<td>Context discrimination</td>
</tr>
<tr>
<td>Contextual fear (CFC)</td>
</tr>
</tbody>
</table>

↑ Increase- results from current thesis
↓ Decrease- results from current thesis
↑ Increase- known from literature
× Affected- results from current thesis
↓ Decrease- expected result

![neuronal firing]

ripple activity

place cell activity

Figure 8.1 Effects of chronic stress on CA1 neuronal network activity and hippocampus-amygdala mediated behaviours.
Discussion

8.1 Suppressed CA1 pyramidal neuronal firing during the exposure to immobilization stress- in vivo recordings

Population comparison of neuronal firing during first baseline activity period (Rest) to during immobilization period displayed suppressed neuronal activity throughout the 2h (Chapter-3 and 4). The variance (Inter-Quartile-Range) in firing rates of CA1 neuronal population though exhibited a decrease during Last-30’ as compared to First-30’.

One possible explanation for the significantly low population firing rates during immobilization, as compared to immobilization stress, could be elevated neuronal firing during Rest as mice entered the recording apparatus for the first time. Environmental novelty has been previously reported to cause enhancement of CA1 neuronal firing (Wilson and McNaughton, 1993; Wilent and Nitz, 2007; Karlsson and Frank, 2008). In fact, the observation that control animals also display a decrease in neuronal firing (though insignificant), points towards this possibility. Thus a combination of elevated firing during Rest and decrease in variance during immobilization appears to provide a significant difference between these two behavioural states. The observation that CA1 neuronal firing does not display a difference between First-30’ and Last-30’ is in agreement with two previous studies that employed corticosterone (CORT) as a proxy for the presence of a stressor (Barak et al., 1977; Yamato et al., 2002).

An alternative interpretation for suppressed CA1 firing during immobilization stress could be that the unavailability of any visual or idiothetic cues as the animal is immobilized in a polythene bag. In fact, studies that blocked the self motion cues (Foster et al., 1989; Gavrilov et al., 1998; Stackman et al., 2002), information processing of self motion cues (Russell et al., 2003) or made the spatial cues unavailable by making the rats blind (Save et al., 1998), did observe a decrease in CA1 neuronal firing.

Therefore, from the above data we can only conclude that immobilization does not cause an enhancement of CA1 neuronal firing. Future studies need to look at the role of inhibition in this suppressed CA1 pyramidal cell firing during immobilization. For example, suppressed firing of CA1 inhibitory neurons will support the view that cessation of inputs cause the suppressed neuronal firing. However, enhanced firing of CA1 inhibitory neurons will refute this hypothesis.
8.2 Enhanced CA1 ripple activity during the exposure to immobilization stress- *in vivo* recordings

In view of the fact that stress impairs spatial memory, next the sharpwave-associated ripple activity that has been implicated in spatial memory (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010; Jadhav et al., 2012) was examined during immobilization stress. Compared to Rest activity, ripple amplitude and length displayed an enhancement during immobilization but not during exploration (Chapter-3 and 4). Furthermore, these results were further confirmed by comparing the ripple activity during immobilization to the 2 h data from Control animals during the Rest period.

The most simplistic explanation of enhanced ripple amplitude and length could be that somehow during immobilization, the tetrodes have physically moved closer to the source of ripple generator. The maximum ripple amplitude is observed in the pyramidal cell layer (O’Keefe and Nadel, 1978; Buzsáki et al., 1992). Before a recording session, we made sure that tetrodes were placed in the cell layer so that we could get maximum number of neurons for analysis (see Chapter-2). Thus movement of tetrodes could only give rise to a decrease in amplitude not an increase as observed in our experiments. Thus enhanced ripple amplitude appears to be a real phenomenon. Enhanced ripple length could be caused by a decrease in background noise, as this will allow the detection of sinusoidal fluctuations in CA1 network (LFPs) for longer duration. Greater synchronization between neurons has been reported to be observed during ripple activity (Chrobak and Buzsáki, 1996; Csicsvari et al., 1999). Thus suppressed neuronal firing, but possible enhancement of CA1 neuronal co-activity during immobilization can give rise to larger amplitude as well as greater ripple length. However, this hypothesis remains to be tested.

Greater ripple amplitude has been reported to occur after associative learning (Eschenko et al., 2008). From this point of view, animals may be consolidating the information learnt prior to stress. Since we did not perform the spatial learning paradigm, it is difficult to comment on it further. Noticeably, the information content (a parameter of place cell activity) of CA1 place cells from Stress group displayed a slightly greater increase than Control group during repeated exposure to a linear-track (Chapter-5).

An obvious analysis that remains to be done is to examine if co-activity of CA1 pyramidal neurons increases during immobilization. Furthermore, because ripple activity are reported to share similar mechanisms (Sullivan et al., 2011) with gamma rhythms, another high frequency rhythmic activity, it will be worth analysing the power of gamma rhythm during the immobilization period.
8.3 CA1 place cells from the Stress group fail to modulate their firing or field size - in vivo recordings

Hippocampal neurons are reported to encode spatial and contextual information by modifying their firing rates as well as the preferred location of enhanced firing. To assess the effects of stress on spatial encoding, I recorded place cell activity from stressed rats at multiple time points. An important observation from this analysis was that stressed animals failed to display changes in firing rates as well as field size on two different linear tracks (Chapter-5). This inability to modulate the population place cell activity in stressed animals thus indicates deficits in spatial encoding.

Firing rate rigidity in two environments that share the spatial cues has previously been observed in aged animals, though in sub region CA3 (Wilson et al., 2003, 2004). In addition, 35-40% of place cells in aged animals fail to encode changes in the spatial cues in the environment (Tanila et al., 1997). Furthermore, if an animal is taken out of a familiar environment, put in a novel environment and again put back into the same familiar environment (familiar-novel-familiar), unlike young rats, aged rats do not exhibit the same representation in the familiar environment, as they had shown earlier (Barnes et al., 1997). Based on these results, aged animals are reported to display a failure to use subtle changes in cues (partial pattern-separation deficits). In addition, when they come across very different environments, they can indeed make different cognitive maps for these environments. However, now if they re-experience the familiar environment, they have difficulty accessing the old maps (partial pattern-completion deficits). Thus it is possible that similar to aged animals, stressed animals also display deficits in pattern completion/separation. However, this possibility needs further investigations.

Lynn Nadel and colleagues have suggested that hippocampal place cells can encode more than space (Nadel and Willner, 1980). According to Nadel and colleagues, hippocampus being an associative cortex should be able to associate multimodal cues and spatial cues happen to be one of the types. This way of thinking has led to the hypothesis that hippocampus is involved in contextual encoding. In fact, CA1 place cells do change their firing rates across various geometrical and non-geometrical features such as colour, odour, texture, and time intervals (Bostock et al., 1991; Anderson and Jeffery, 2003; Deshmukh and Bhalla, 2003; Wilson et al., 2004). Thus rigidity in firing rates and field size of CA1 neurons in spite of change in texture, colour and geometry of the linear tracks, points to impaired contextual coding in stressed mice. It may not be far-fetched to assume that impaired pattern completion/separation function caused by structural and synaptic plasticity impairments in stressed animals thus give rise to context generalization in stressed animals.
But then what can cause the inability to change firing rates or field size across two tracks on either D6 or D11? CA1 neuronal firing can be affected by changes in area CA3. In addition, the entorhinal cortex (EC) that provides inputs to the hippocampus can also affect CA1 place cell activity. Firing rate rigidity across spatial context may well be the result of poor spatial encoding upstream of the hippocampus. Thus future studies should assess the CA3 place cell activity and EC grid cell activity in the context of stress.

8.4 Chronic stress causes enhancement of contextual fear learning, especially if exposure to the context was short during conditioning-behavioural experiments

We observed that the extent of freezing during fear recall is a function of the contextual exposure time that a rodent received during the previous (Conditioning) day. Control animals that received a foot-shock immediately after placement into the context (short PSI); displayed very less freezing during context recall. A longer exposure to the context caused a higher freezing response during recall in these rats. This result of correlation between freezing (CR) and exposure time to the context (CS duration), in control animals is in agreement with previous literature (Fanselow, 1990; Bevins and Ayres, 1995; McHugh and Tonegawa, 2009). Interestingly, chronic stress caused a change in the slope of this function such that while peak did not change the slope did exhibit a change (Chapter-6).
This result is bit puzzling, as how can spatial memory be impaired while context learning is enhanced? It must be noted that our observation of higher context fear is in agreement with earlier studies that have assessed the effects of stress on CFC (Conrad et al., 1999; Sandi et al., 2001; Cordero et al., 2003). It has been suggested that during short PSIs, the hippocampus does not get enough time to make conjunctive representations of various contextual cues (Rudy and O’Reilly, 1999). Thus, stress appears to have strong effects on contextual fear learning in the scenario where the hippocampus is unable to encode conjunctive representations.

![Figure 8.3 Chronic stress lowers the threshold for fear acquisition.](image)

Though the CFC paradigm is commonly employed to probe hippocampal function, it is not an exclusively hippocampus-dependent task (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Maren et al., 1996a). Instead, it is dependent on hippocampus-amygdala circuitry (LeDoux, 2003; Alvarez et al., 2008). Thus, the amygdala may also contribute to the enhanced context fear phenotype observed in stressed rats. In fact, accumulating data from our lab suggests that chronic stress enhances the structural and synaptic plasticity in the basolateral amygdala (BLA), a subnuclei of the amygdala involved in context-foot-shock (US) association (Vyas et al., 2002; Mitra et al., 2005; Lakshminarasimhan and Chattarji, 2012; Ghosh et al., 2013). Chronic stress induced changes in the BLA have been hypothesized to enhance the output function of the amygdala. Thus it is possible that this contribution of the amygdala causes enhanced context fear in the CFC paradigm while no change is observed in other spatial learning paradigms that exclusively depend on the hippocampus.

This hypothesis needs to be tested. A set of experiments involving a context familiarization task and a context fear discrimination task should be able to provide more light on this issue. It is expected that stressed animals will display enhanced generalization on any task that will need
foot-shock association while they may display poor familiarity and/or discrimination in the absence of an aversive association.

8.5 Chronic stress affects time interval coding in one-trial contextual fear conditioning-behavioural experiments

It has been shown that at short intervals animals remember better. Animals are able to learn the longer time intervals but the error grows in a scalar manner (Gibbon, 1977; Gibbon et al., 1984). Employing four different time-intervals between placement of rats into the conditioning arena and foot-shock delivery (Placement to Shock Interval or PSI), we subsequently tested a stressed animal’s ability to remember the context and time intervals. Control animals displayed maximum freezing at the min when they had received a foot-shock in the context the previous day. At short exposure all the animals displayed freezing within 1st min while for longer PSIs the curve was broader (Chapter-6). In agreement with previous rodent studies, we found a linear relationship between preferred freezing min and time interval (King et al., 2001; Buhusi et al., 2009). While these studies often use an animal trained on multiple intervals, our experimental design did not allow for this. However, we do see a preferred peak time interval in the rat population, which approximately corresponds to the time-interval they received between placement and shock interval (PSI) during single trial conditioning the previous day. Interestingly, this anticipatory freezing at the correct PSI appears to get affected in stressed animals. Somehow they appear to show peak freezing at 3rd min bin.

![Fig 8.4 Chronic stress affects interval-coding in one-trial context fear conditioning.](image-url)
At present we have no explanation for why this should happen. The hippocampal neurons (Time cells) have been recently reported to display enhanced spiking at specific moments during a period (MacDonald et al., 2011; Kraus et al., 2013). It appears that chronic stress may impair the activity of hippocampal time cells. In addition, extra-hippocampal areas may also affect the functioning of time cells.

Our results of impaired encoding of time interval is at odds with the only study in our knowledge that has studied the effect of stress on time interval (Leuner et al., 2004). This study found enhanced trace fear conditioning that is dependent on the hippocampus for remembering a time trace between cessation of a conditioned stimulus (CS: say auditory tone) and delivery of an unconditioned stimulus (US: say foot-shock). However, this study used a trace eyeblink conditioning protocol that used 300 pairings CS-US pairings per day for 2 days which cannot be compared with current experiments. In fact, trace conditioning at such millisecond intervals is reported to be hippocampus independent (Moyer et al., 1990) and it is only at longer exposure of 20-30s or more that the dorsal hippocampus plays an important role (Chowdhury et al., 2005; Quinn et al., 2005).

While comparing the data across groups we show that time-interval coding is affected by stress. The next set of experiments should employ different intervals in the same animals and test and repeat the same experiments. The deficits in accuracy of time estimation observed in stressed animals, is also a common feature in various disorders in human patients ranging from Attention Deficit Hyperactivity Disorder (Barkley et al., 2001; Gilden and Marusich, 2009), Alzheimer’s disease (Nichelli et al., 1993) to Schizophrenia (Densen, 1977; Penney et al., 2005). In light of this observation, single-trial contextual fear conditioning experiments can be used in screening the animals for various stress disorders (King et al., 2001).

8.6 Stress facilitates acquisition of amygdala-mediated auditory fear conditioning

Accumulating evidences from our laboratory suggest that chronic stress creates an ideal synaptic substrate for future amygdala-mediated learning (Vyas et al., 2002; Mitra et al., 2005; Vyas et al., 2006; Lakshminarasimhan and Chattarji, 2012; Ghosh et al., 2013). This hypothesis was tested by employing auditory fear conditioning (AFC), a Pavlovian learning paradigm that needs an intact amygdala (Selden et al., 1991; Phillips and LeDoux, 1992; Sananes and Davis, 1992). In agreement with the above hypothesis, stressed animals displayed enhanced freezing response during tone recall (Chapter-7). We also observed that stressed animals did not display enhanced freezing if conditioned with a high foot-shock intensity protocol. It was observed that strong-foot shock gave rise to a ceiling effect such that Control rats themselves display high freezing and stress could not further increase the freezing at strong foot-shock protocol. The comparable freezing response between stressed animals with weak foot-shock conditioning and
control animals with high foot-shock conditioning thus suggests that stress lowers the threshold for the formation of auditory fear memory. Furthermore, it can also be concluded that strong foot-shock conditioning is stressful. My experiments only assessed the short-term effects of chronic stress on AFC. It is yet to be studied if these results persist for longer.

8.7 Similarities and contrast between chronic stress and aging (table 8.1) – amygdala dominance decides the freezing during contextual fear conditioning

This section of the discussion is prompted by the similarities observed between the inability to modulate place cell activity in stressed animals (my observations from chapter-5) and in aged rats(Wilson et al., 2005). Wilson and colleagues had observed the similar phenotype, though in area CA3. They hypothesized that rigidity in firing rates of place cells may be the mechanism underlying spatial learning deficits observed in aged rodents (Barnes, 1979; Deupree et al., 1993; Gallagher et al., 1993). They called this inability of aged animals to modulate firing rate across different spatial contexts as cognitive aging. It should be noticed that our study focused on CA1 place cells and the impact of chronic stress on in vivo CA3 neuronal activity remains unknown.

Parallels between neuronal firing rate rigidity, and spatial learning deficits in stress and aging prompted us to ask if synaptic plasticity and associative learning paradigms such as contextual fear, temporal coding, and auditory fear conditioning will also display an enhancement in older rodents. During the course of this literature survey we found that indeed stress and aging both share common mechanisms and there exists a glucocorticoid hypothesis of stress and aging (Sapolsky et al., 1986). At least one human study has provided evidence in favour of this view (Lupien et al., 1998).

In agreement with stress data, aged rodents display impaired hippocampal synaptic plasticity such that the threshold for generation of LTP is raised in aged animals (Landfield et al., 1978; Norris et al., 1996; Foster, 1999). Furthermore, LTP decays much faster in old animals (Landfield et al., 1978; Barnes, 1979) while the threshold for the generation of LTD is lowered(Norris et al., 1996; Foster and Norris, 1997). Furthermore, similar to stress, aging also causes a decrease in dendritic length (Markham et al., 2005) and a smaller number of dendritic spines on CA1 neurons (von Bohlen und Halbach et al., 2006; Luine et al., 2011). Interestingly, aging had mixed effects on various associative learning paradigms. For example, while some studies indicated a decrease in contextual fear in old animals (Houston et al., 1999; Ohta et al., 2001; Moyer and Brown, 2006), others failed to find a difference in CFC paradigm between young and old animals(Gould and Feiro, 2005; Woodruff-Pak et al., 2010). Similarly, while some studies reported impaired trace fear conditioning (Moyer and Brown, 2006), some others did not observe the same. Thus not much can be concluded from above data except that aged
animals do not display enhancement of hippocampus-mediated associative learning paradigms. In fact cued fear conditioning that is mediated by the amygdala was impaired in some cases (Gould and Feiro, 2005) while no change was observed in another study (Ohta et al., 2001). A summary of above literature survey is presented in table 8.1

Table 8.1 Effects of chronic stress and aging on hippocampus at different levels of neural organization.

<table>
<thead>
<tr>
<th>Aging</th>
<th>Behaviour</th>
<th>Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spatial memory</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Contextual fear (CFC)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Context discrimination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trace conditioning</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interval timing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cued fear (AFC)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Generalized fear (anxiety)</td>
<td></td>
</tr>
</tbody>
</table>

Network (in vivo) - Hippocampus

<table>
<thead>
<tr>
<th>Chapter-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firing rate</td>
</tr>
<tr>
<td>Rate remapping</td>
</tr>
<tr>
<td>Place field size</td>
</tr>
</tbody>
</table>

Neuron/Synapse - Hippocampus

<table>
<thead>
<tr>
<th>Chapter-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTP</td>
</tr>
<tr>
<td>LTD</td>
</tr>
<tr>
<td>Dendritic length</td>
</tr>
<tr>
<td>Neurogenesis</td>
</tr>
<tr>
<td>Spines</td>
</tr>
</tbody>
</table>

Symbol Key:
- Increase-known from literature
- Decrease-known from literature
- No change-known from literature
- Affected-known from literature
* Threshold for LTP induction enhanced and LTD decays faster known from literature
** Threshold for LTD induction is lower known from literature

Two symbols together indicate that changes in behaviour is observed in both directions.
Overall it appears that any associative learning paradigm that has a fear component involved does not display an enhancement in aged animals. This was contrary to what we had expected. A further literature survey found occasional reports of impaired amygdala activity (Herzog AG, 1980) as well as impaired amygdala-hippocampus circuitry (Almaguer et al., 2002). Thus impaired influence of amygdala on the hippocampus during aging is associated with impaired contextual and trace fear learning. However, hyperexcitability of amygdala, and its greater influence on the hippocampus (Ghosh et al., 2013) is associated with an enhanced contextual
fear, trace fear and enhanced cued fear learning. Such parallels with direction of influence of the amygdala on the hippocampus; and the contextual and trace fear learning thus provides support the assumption that the amygdala strongly influences the aversive associative learning (Fig 8.5).

Conclusions and future directions

In this thesis, we began with CA1 neuronal recordings. We found that stressed animals were unable to modulate their firing rates and place field size across two different spatial contexts. We then used one trial contextual fear learning and observed that stress enhanced contextual fear while anticipatory behaviour was also affected. Furthermore, stress also enhanced amygdala-mediated auditory fear conditioning and anxiety. Based on these results we hypothesize that it is the enhanced amygdala hyperactivity that causes enhanced fear. We also speculated that enhanced contextual fear should not be context specific but generalized. Furthermore, we compared all these results to aging studies, as both stress and aging share common mechanisms (Sapolsky et al., 1986; Sapolsky, 1992; McEwen, 1999). A literature survey then suggested that hippocampus-mediated spatial learning and synaptic plasticity is impaired in both stressed and old animals. Furthermore, the enhanced amygdala activity in stressed animals was paralleled with enhanced contextual and trace fear learning. Similarly impaired amygdala activity in aged animals was observed along with impaired contextual and trace fear conditioning. From the above two observations, we deduced that contextual and trace fear conditioning paradigms, since they use an aversive stimulus (foot-shock, US), should be considered as function of hippocampus-amygdala circuitry rather than hippocampal behaviour as very often used in the literature.

Below are some of the directions that can be followed to get a better understanding on the effects of stress on hippocampal functionality.

• Role of chronic stress on CA1 inhibition, especially on the firing of perisomatic PV+ve inhibitory neurons.
• The effects of chronic stress on CA3 place cell activity.
• Long term effects of stress on CA1 place cell activity and CFC.
• It is yet to be experimentally determined if the spatial and contextual discrimination is indeed affected in chronically stressed animals.
• Effects of chronic stress on various levels of neural organization in EC needs to be studied
Furthermore, since place cell firing can be modulated by either the amygdala (Kim et al., 2001; Ghosh et al., 2013) or the entorhinal cortex (Brun et al., 2002, 2008), we propose that future studies should also focus on simultaneous recordings from CA1, MEC and BLA.

Figure 8.6 The basic circuitry diagram of hippocampus-amygdala-entorhinal connectivity. The effects of chronic stress on CA3 plasticity needs to be examined. The impact of stress on subiculum and entorhinal cortex have been overlooked. Thus role of stress at various levels of neural organization needs to be studied in subiculum and entorhinal cortex. Dentate gyrus (DG), Entorhinal cortex (EC). Downside arrow depicts a decrease while upside arrow indicate an increase. Question mark indicates that currently no information is available.