Atrophy in the medial prefrontal cortex as a consequence of chronic stress

The role of the basolateral amygdala

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Abstract

It is known that chronic stress has aversive effects on the prefrontal cortex and especially on the medial prefrontal cortex (mPFC). Several studies have consistently shown that pyramidal neurons in this area are affected. In specific, atrophy occurs on apical spines and dendrites of these neurons. Chemical key players herein are glutamate and corticosterone. Importantly, there are indications that the basolateral amygdala (BLA) plays a role in this. Anatomical and electrophysiological studies reveal that the BLA has glutamatergic projections that are widely distributed over the mPFC. Projections that might play a role in chronic stress-induced neuronal atrophy are direct, monosynaptic projections and indirect, polysynaptic projections. A prominent type of interneurons that is part of the indirect projection is the parvalbumin (PV) interneuron. It turns out that this group of interneurons is affected by chronic stress. Although scarce, there is evidence that restoring PV interneurons can prevent stress induced neuronal atrophy. In addition, there is evidence that glutamatergic output from the BLA is dysregulated after chronic stress. A further role for the BLA in neuronal atrophy of the mPFC with regard to glucocorticoids is confirmed in lesion studies. Studies suggest that the BLA has an important role in neuronal atrophy in the mPFC via regulating the glucocorticoid response and its receptor expression. In general, these findings suggest an important role for the BLA in the effect of glutamate and glucocorticoids in neuronal atrophy in the mPFC. However, direct correlations between the extent to which an area in the mPFC is innervated by glutamatergic BLA-projections and neuronal atrophy have not yet been studied. In addition, it seems that studies on PV interneurons in relation to neuronal atrophy in this area in particular still need to be done, despite evidence on other brain areas.

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0. Introduction

Stress is an intriguing phenomenon. It can be defined as a complex response of the body to an overwhelming situation so that the individual becomes more capable to cope with that situation on the longer term (1). For example, a certain level of stress makes us able to deal with the current situation of a lock down to hamper the spread of the coronavirus. However, it is commonly known that chronic stress has multiple deleterious effects on several bodily and mental processes.

For instance, of several brain regions, it is reported that they undergo structural alterations as a consequence of stress and in particular chronic stress. There is also ample evidence that structural changes occur in regions of the hippocampus, amygdala and prefrontal cortex (PFC) following chronic stress. Elaborating on the latter brain region, it is often observed that especially the medial prefrontal cortex (mPFC) is altered in its neuronal structure. In particular, it was observed multiple times that chronic stress induced neuronal atrophy in this region (2–4).

Now, there are reasons to think that especially the basolateral nucleus of the amygdala (BLA) plays a role in the neuronal atrophy that occurs in the mPFC as a consequence of chronic stress. First of all, an altered glutamatergic tone in the mPFC is associated with this atrophy (5). Secondly, there are indications that glutamatergic neurotransmission between the BLA and mPFC is altered following chronic stress (6). In addition, there is evidence that lesioning or inactivation of the BLA prevents an increased expression of glucocorticoid receptors in the mPFC (7). Of importance is that atrophy of the mPFC is associated with activation of these receptors by stress hormones (8, 9). Taking the above-mentioned key points into consideration, it is indeed likely that the BLA is important in atrophy of the mPFC. Based on these insights, we try to further elucidate the relationship between the BLA and, more specifically, neuronal atrophy on pyramidal neurons in the mPFC.

In this review, the neuronal atrophy in the medial prefrontal cortex (mPFC) as a consequence of chronic stress is discussed in detail, together with the possible role of the basolateral amygdala (BLA) in this process. How and at what part of the pyramidal neurons does the atrophy occur? What are the possible mechanisms that underlie this atrophy? Thereafter, the 'connectivity' between the BLA and mPFC is reviewed. In this case, this concept of connectivity is partitioned into neuroanatomy and neurotransmission. How does the BLA innervate different areas in the mPFC and what kind of neurotransmission occurs on the 'BLA-mPFC' pathway? Consequently, the effect of chronic stress on this will be considered. In other words, how does the neurotransmission in this pathway alter after subjection to chronic stress and what is the possible role of this altered neurotransmission on neuronal atrophy in the mPFC? Lastly, the role of the amygdala in this will be discussed from the perspective of lesion studies. In particular, how do these lesions influence the neuronal atrophy following chronic stress?

1. Neuronal atrophy of pyramidal neurons in the medial prefrontal cortex as a consequence of chronic stress

1.1. Significant atrophy of distal dendrites and spines occur mainly on the apical side of pyramidal neurons

In the neurosciences, stress paradigms are used to assess the effect of stress on a certain brain region of interest. Two widely used forms of stress that are used in such experiments are acute stress and chronic stress. Here, we mainly focus on the effects of chronic stress, since it is proven that this form of stress has a profound effect on several brain areas. A good example thereof is the medial prefrontal cortex (mPFC).

To date, numerous studies have assessed the effect of chronic stress on the neuronal structure of the mPFC. It was often found that chronic stress resulted in significant atrophy of the mPFC. Now, there is already a decent body of research that has been done on aversive effects of chronic stress on the mPFC in animal models (2, 3, 10–14). The main aversive effect of chronic stress mentioned in research on this topic is the atrophy of the mPFC. Here, atrophy is defined as the loss of volume of a specific brain area. An underlying reason for this is the shrinkage of neuronal structures.

To start with, several stress paradigms were applied to assess the influence of chronic stress on architecture of the mPFC. The duration of the procedures varied from two to six weeks over several studies and stressors were applied for several hours per day. Different forms of chronic stress were applied. For instance, these include variable, restraint and unpredictable chronic stress. Firstly, restraint stress amounts to the limiting of the ability to move freely. Secondly, in the case of variable stress, more than one stressor was applied at one day. Unpredictability of stress was brought about by randomly distributing the several stressors over different times of the day (3, 10). A single stress paradigm can entail multiple stressors. Examples of stressors here are tail suspension, forced swimming, limited ability to move freely (restraint) and exposure to extreme temperatures in rats (2, 4, 10, 13).

Especially in the case of aversive effects of chronic stress on the mPFC, it is the shrinkage of dendrites in this brain area (2–4, 10–13). The mPFC can in turn be subdivided into the infralimbic region (IL), the prelimbic region (PL) and the anterior cingulate gyrus or cortex (ACC/ACg). In all three regions, atrophy of pyramidal neurons in layer II/III of the mPFC was repeatedly reported after chronic stress (2, 4, 10, 13). Globally seen, the stress-induced atrophy affected as well dendrites as dendritic spines, but the latter one to a lesser extent (2, 3). For example, the dendritic atrophy ranged from 20 to 50 percent, depending on the brain region that was assessed on this (3, 10, 13, 14). On the contrary, the dendritic spine loss ranged from 10 to 25 percent in the studies that were assessed (2, 4, 10, 11). In particular, several studies emphasize that most of the structural alterations happened on the apical side of pyramidal dendrites, which is reflected by the fact that significant reductions in dendritic length or material were only found on the apical side and not on the basal side of the pyramidal neurons (2, 3, 10, 12, 14).

Moreover, there were also certain patterns found with respect to the site at which this deterioration in structure took place along the apical side of pyramidal neurons. A particular method to analyze this is

called a Sholl analysis. With this, any form of dendritic material, such as number of or length of dendrites, is measured as a function of the distance from the soma (15). Here, an intersection can be defined as the point at which a sprouting dendrite and the main arbor intersect. Remarkably, a reduction of intersections can be seen at more distal parts of the apical side, which was also found in several other (3, 4, 10, 12, 13). This means that less dendrites are sprouting from the main arbor, which can be interpreted as dendritic atrophy. However, in some cases this deviated towards more proximal parts of the soma (10, 14), suggesting that chronic stress leads to a somewhat general atrophy of the dendritic material on the apical side, but still accentuated on the distal parts of the dendritic arbor. In the right half of *figure 2*, an altered neuronal structure can be seen as a result of chronic stress. One can see a shrinkage of apical dendrites and general impoverishment of the cytoarchitecture of the neuron that is depicted centrally.

As mentioned before, the atrophy of the mPFC is also accompanied by the loss of spines. Especially distal spines are severed by chronic stress (2, 4). Degradation of spines that are situated on more than 200 µm from the soma was reported (2, 4). Now that we have a picture of how atrophy in the mPFC occurs, we can move on to mechanisms that might underlie this structural deterioration.

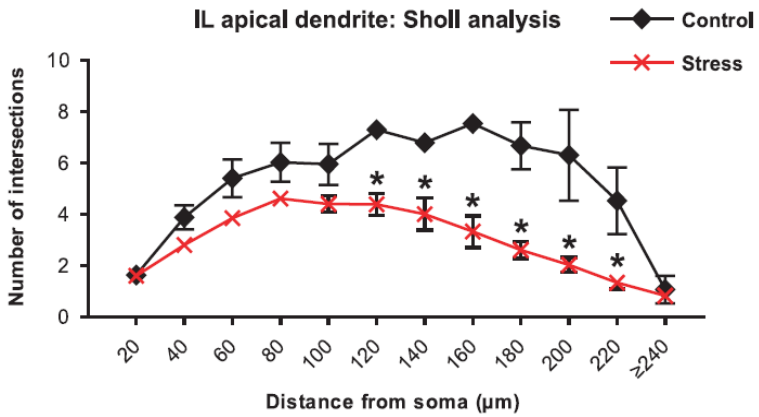

Figure 1. A Sholl analysis of apical dendritic material in the infralimbic region of control rats and chronic stressed rats. The number of intersections is measured as a function of the distance from the soma (from Dias-Ferreira et al. (3)).

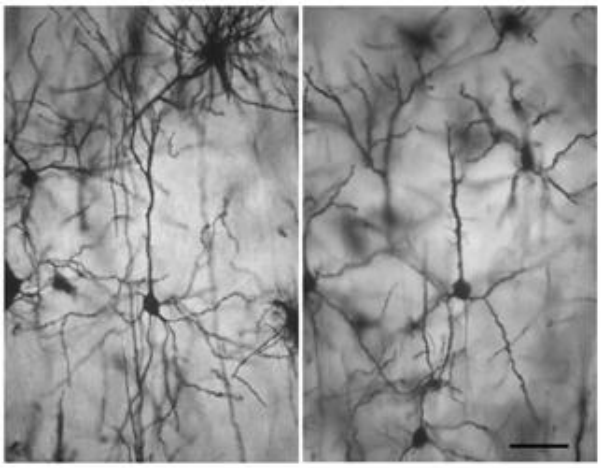

Figure 2. Digital light micrographs of Golgi-stained neurons in layer II–III of medial prefrontal cortex in an unstressed (left) and stressed (right) rat. (from Cook & Wellman (12))

1.2. Glutamate and glucocorticoids may play a role in the atrophy of the mPFC following chronic stress

In general, it has been shown that there are several underlying mechanisms that contribute to the apical dendritic atrophy in the mPFC following chronic stress. In other words, how does the formerly discussed atrophy come about and why on such a specific site of the pyramidal neurons? To begin with, it has been demonstrated that chronic stress is associated with an increase in glutamatergic neurotransmission in the mPFC (16). This is supported by earlier observations in relation to atrophy in the mPFC. Namely, it Jiang et al. (5) showed that excitotoxic effects of glutamate followed substantial loss of dendritic spines. After all, the loss of dendritic material was most profound on the apical side of the pyramidal neurons studied. Together with Jiang's finding (5) on the excitotoxicity of increased glutamatergic neurotransmission, there is also evidence that apical dendrites of pyramidal neurons are densely endowed with glutamate sensitive NMDA R2B receptors (17). On top of that, it was shown that these receptors play an important role in corticosteroid-induced excitotoxicity (18). Thus, these findings might support the numerous observations that dendritic atrophy following chronic stress is limited to the apical region of the pyramidal neurons. This may be due to an elevated glutamatergic neurotransmission onto this side of the neurons.

Importantly, in several experiments regarding chronic stress, corticosterone was measured as a correlate of chronic stress (4, 13, 19). This could mean that the glucocorticoid corticosterone is also involved in dendritic atrophy. As mentioned earlier, glutamatergic excitotoxicity is induced by corticosteroids. This idea is further supported by observations that the increase in glutamate that goes along with stress was blocked by the administration of a glucocorticoid receptor (GR) antagonist, meaning that the glutamate response is indeed induced by activation of this GR (8). Zooming in further on the effects of glucocorticoids, there is a need for coming back on the study of Lopes et al. (10). In this study, an important interaction between the tau protein and glucocorticoids was observed. The tau protein is an essential protein for the assembly of microtubules that are in turn important for the maintenance of cytoarchitecture of neurons (20, 21). In their study, they showed that mice lacking the protein tau did not suffer from dendritic atrophy in the mPFC, whilst having similar corticosterone responses to stress. This suggests an important role of tau in the observed dendritic atrophy (10). This is further supported by evidence that glucocorticoids triggered tau hyperphosphorylation in a rat neuronal cell line (9). Thus, these experiments indicate an important role for glucocorticoids in neuronal atrophy that is possibly mediated by tau (see *figure 3*).

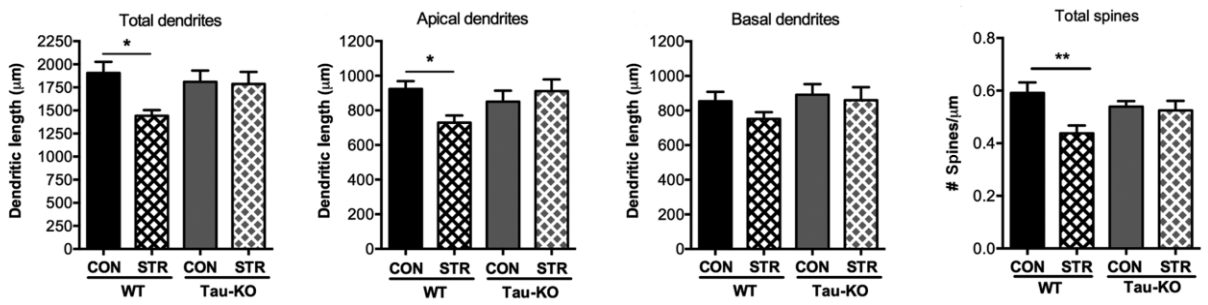

Figure 3. Measurements of dendritic length and spine number in control and stressed wildtype mice and control and stressed mice lacking tau (edited from Lopes et al. (10)).

Concludingly, chronic stress has a deleterious effect on the integrity of the mPFC. Two chemical key players, glutamate and glucocorticoids, seem to play a role in the dendritic atrophy on the apical side of pyramidal neurons in the mPFC. As mentioned in the introduction, there are indications that the basolateral amygdala (BLA) is involved in the atrophy of the mPFC after chronic stress, regarding glutamate and glucocorticoids. Firstly, in several studies, it has been established that there are afferents going from the BLA to the mPFC and that these afferents are glutamatergic. Importantly, these afferents seem to be dysregulated in the situation of chronic stress. Secondly, lesion studies have revealed an important role for the BLA in regulating levels of glucocorticoids, their receptors and in the atrophy of the mPFC. In the next chapter, we will focus on the neuroanatomy going from the BLA to the mPFC. What are the properties of the BLA-mPFC projections and how could this contribute to atrophy in the mPFC?

2. Properties of the projections from the BLA to the mPFC

Already for a long time, studies on the interconnections between the BLA and mPFC have been done. Numerous (reciprocal) connections between the BLA and mPFC have been elucidated already. Here, we show which regions of the mPFC - that undergo atrophy following chronic stress - are innervated by the BLA. Furthermore, we will study which type of neurons are innervated. In addition, we will look what type of neurotransmission occurs on this BLA-mPFC pathway, from the perspective of immunohistochemistry and electrophysiological studies. This is important, since neurotransmission systems might contribute to neuronal atrophy in the mPFC, as we have discussed in the previous chapter.

2.1. The BLA has distinct projections towards the mPFC that are diffusely distributed over several layers of the mPFC

Here, it is discussed which connections there are that originate from the basolateral amygdala (BLA) and go towards the medial PFC (mPFC). These connections were investigated in several studies (22–26). Methods widely used to figure these connections out are retrograde and/or anterograde tracing. With

retrograde tracing, it can be assessed which afferents a part of the mPFC can have, by injecting a fluorescent tracer into the concerning part of the mPFC. Conversely, in the case of anterograde tracing, injections into the BLA of a fluorescent dye were made to assess which cortical regions in the mPFC were innervated by this region (22–26). With these methods, several pathways going from the BLA towards the mPFC were unraveled.

Thus, it was revealed that the BLA has innervations in distinct parts of the mPFC. For example, areas in the mPFC that are innervated are the infralimbic area (IL), prelimbic area (PL) and anterior cingulate cortex (ACC) (22–26). Besides, there are also different layers in these subdivisions that have afferents from the BLA. These include layers 2 through 6 of the mPFC, indicating that the BLA diffusely innervates the mPFC over a wide range of cortical layers (27, 28).

On top of that, the BLA-mPFC projections can also be subdivided into two groups. Initially, we have the direct connections that go from the BLA towards pyramidal neurons in the mPFC (28, 29). However, there are also indirect connections, meaning that BLA-projection neurons innervate intracortical interneurons in the mPFC that are also widely distributed across several cortical layers in the mPFC (30). These intracortical interneurons in turn innervate pyramidal neurons in the mPFC (29).

Remarkably, it is at least known that atrophy in the mPFC occurs in layer II/III of pyramidal neurons. Given that the BLA entails these layers with respect to the innervation of the mPFC, it could be that the BLA-mPFC connections play a role in the decline of the neuronal structure in the mPFC. In the next sections, we will look more closely at the properties of these connections. Interestingly, we will find that there are indications that these projections have a glutamatergic component, keeping in mind that glutamate seems to play a major role in the neuronal atrophy in the mPFC.

2.2. The direct projections in the BLA-mPFC pathway are glutamatergic and project towards the spines upon the pyramidal neurons in the mPFC

Regarding the direct or ‘monosynaptic’ projections, the BLA mostly innervates the spines upon the aforementioned cortical pyramidal neurons. These include not only spines on the apical side, but spines on the basal side of the pyramidal neurons as well (28). Earlier in this review we discussed that atrophy of spines occurs after chronic stress and that glutamatergic neurotransmission plays a role in this. Interestingly, there are several indications that the direct, monosynaptic projections are glutamatergic. This follows from immunohistochemical and electrophysiological studies. For instance, it was observed that BLA pyramidal neurons showed immunoreactivity for glutamate, implying that these neurons have glutamatergic neurotransmission (31). This is further supported by several electrophysiological studies (32–34).

A paradigm to study electrophysiological properties of BLA neurons is the placement of stimulating electrodes in the BLA and recording electrodes in the PFC. In this way, one can stimulate projectory neurons in the BLA whilst monitoring activity of the recipient, pyramidal neurons in the mPFC (32, 33, 35). To start with, the characteristic of a direct, monosynaptic projection is supported by the observation of a short latency time on average when these neurons are activated, meaning that there is a short duration between stimulation and onset of action potential (32, 34, 35). The excitatory characteristic of this

monosynaptic pathway can be revealed by administering the drug DNQX close to the recording site, the pyramidal neurons of the mPFC. This drug is an antagonist that blocks the receptor that is involved in creating the postsynaptic potential. With this, it was found that the monosynaptic pathway is indeed excitatory (33). More in detail, two receptor systems seem to be involved in these connections. One is the AMPA-kainate ionotropic receiving receptor, since the administration of an AMPA receptor antagonist (DNQX) abolished the evoked excitatory extracellular field potentials. The other receptor, an NMDA-receptor is also shown to be involved in this excitatory projection, but to a minimal extent (33, 35). Since both the AMPA and NMDA-receptor family are activated by glutamate, the monosynaptic excitatory pathway can be seen as glutamatergic. Later on, we will see that there are indications that glutamatergic neurotransmission on this pathway may be altered as a consequence of chronic stress, keeping in mind that glutamate plays a role in neuronal atrophy.

Besides direct projections, there are also indirect projections. The relevance of the indirect projections is that they seem to be affected by chronic stress and this may play a role in neuronal atrophy. However, before delving into this topic, it is first wise to study neurochemical properties of these connections.

2.3. The indirect projections from the BLA to the mPFC are polysynaptic, GABAergic and inhibitory

As mentioned before, the BLA innervates local-circuit neurons as well. These interneurons form connections with pyramidal neurons in the mPFC and are thus essential for the formation of these indirect BLA-mPFC pathways. Nowadays, already a broad range of interneurons that lay in the mPFC has been discovered. Using immunohistochemistry, the presence of a type of interneurons can be demonstrated. To this end, a brain tissue section is incubated in an antiserum against (calcium binding) proteins that are expressed by the interneurons (30). Examples include calretinin, calbindin, somatostatin and parvalbumin interneurons, etc. (30, 36). However, the latter type of interneurons are the highest in their prevalence (30, 37) and have been studied multiple times in the context of chronic stress and even in neuronal atrophy (38–41). For these reasons, we focus on their characteristics and how they function in the BLA-mPFC pathway henceforth.

As with the direct connections, indirect connections have been assessed on their neurotransmission as well. In specific, it has been established that the pyramidal neurons in the BLA have anatomical connections onto the shafts of parvalbumin interneurons (29, 36). Furthermore, it was shown that PV neurons are practically all GABA-positive (42). Given that by far the most of the BLA projection neurons are glutamatergic (31), it indicates that BLA projections onto PV interneurons might be glutamatergic as well. Electrophysiological studies have elaborated on this and show that this is indeed the case.

For instance, inhibiting postsynaptic potentials (IPSPs) were repeatedly observed in the mPFC when neurons in the BLA were stimulated (32, 34, 35). This seems controversial, since nearly all the pyramidal BLA-projection neurons towards the mPFC are excitatory projection neurons (31). This indicates that the inhibitory pathway is at least not monosynaptic, but rather di- or polysynaptic, which is indeed the case. Namely, it was demonstrated that an IPSP evoked in the mPFC by stimulation of the BLA was blocked by administration of a GABA-receptor antagonist, showing that GABAergic neurotransmission plays a role in this pathway and that this is GABA-receptor mediated. Moreover, these IPSPs were also significantly

reduced by non-NMDA receptor antagonists, indicating that the inhibitory pathway is polysynaptic (35). On top of that, after stimulation of BLA-mPFC neurons, the IPSP lagged the EPSP, which is also indicative for a polysynaptic pathway (36). This idea of a polysynaptic pathway is further supported by other evidence. For instance, responses of PV interneurons were explicitly recorded after BLA-stimulation. BLA-stimulation evoked short-latency excitation in all interneurons that were recorded (34). Given that most of the pyramidal neurons were inhibited, this indicates that there is a mechanism of feedforward inhibition in local circuits in the mPFC that regulates the amygdalar output (34). Thus, it seems that PV interneurons are crucial in establishing the inhibitory polysynaptic pathway. In the next chapter, we will see that these PV interneurons are affected as a consequence of chronic stress and that this may contribute to neuronal atrophy, among other findings that could confirm a role for the amygdala in chronic stress.

3. The role of the BLA in stress-induced atrophy of the mPFC

In this chapter, we will discuss possible roles of the BLA in the neuronal atrophy of the mPFC. For instance, chronic stress seems to alter the glutamatergic neurotransmission from the BLA that was discussed earlier. As we will see, the parvalbumin interneurons are also affected by this and might even correlate with neuronal atrophy as well. Another role for the BLA is confirmed from the perspective of lesion studies. Here, the glucocorticoids mentioned in the beginning come by. Namely, it turns out that the BLA can regulate the effects of glucocorticoids on neuronal atrophy of the mPFC.

3.1. Glutamatergic neurotransmission from the BLA is changed after chronic stress and parvalbumin interneurons are severed

There are indications that glutamatergic output from the BLA is altered after chronic stress. A recent and intriguing study of Lowery-Gionta et al. (6) showed that glutamatergic neurotransmission on the BLA-mPFC pathway was indeed altered as a consequence of chronic stress. Interestingly, the change in glutamatergic neurotransmission was opposite in two strains (DBA/2J and C57BL/6J). This was assessed by measuring the paired pulse ratio (PPR), which is inversely related to presynaptic glutamatergic neurotransmission (see also *figure 5*). In the left half of the figure, one can see that stressed mice of the DBA/2J strain show an increase in PPR, meaning a decrease in glutamatergic neurotransmission. As implied earlier, this is the opposite in C57BL/6J mice. However, an important weakness here is that the researchers did not investigate which of the projections from the BLA were contributing the most to this alteration in glutamatergic neurotransmission. Though, there are a couple of studies that imply that the indirect, polysynaptic connection engages in altered glutamatergic neurotransmission from the BLA onto the mPFC as the hitherto discussed interneurons are affected in their activity and number due to chronic stress (6, 38, 41, 43). For instance, exerting an uncontrollable stressor onto mice yields a reduction of excitatory transmission onto PV-interneurons as was observed from electrophysiological recordings of interneurons (41). In addition to altered glutamatergic current, chronic stress can lead to a reduction in

the number of PV-interneurons that was accompanied by a decrease in GABAergic neurotransmission (38), which is consistent with the previously discussed studies that indicated that PV-interneurons are GABAergic (34, 42). Moreover, postsynaptic downregulation of $GABA_B$-receptors is observed in the mPFC (38). Yet, another alteration observed is the decrease in frequency of IPSPs in pyramidal neurons in the mPFC (38, 43), indicating that PV-interneurons are severed by chronic stress. There are reasons to think that this is correlated to neuronal atrophy. Unfortunately, in the studies that were found, neuronal atrophy in the mPFC was not measured in relation to this. Instead, two other brain regions were assessed (40, 44). For this reason, this will be described more elaborately in the discussion section. But first, we will go on to the lesion studies that seem to show a significant contribution of the BLA to neuronal atrophy in the mPFC. In contrast to the studies that we have gone through so far, lesion studies seem to elucidate a role for the glucocorticoid corticosterone that was mentioned in the first chapter.

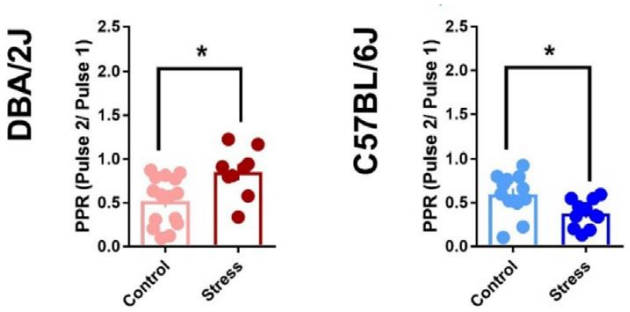

figure 4. Paired pulse ratios (PPR) as a measure of presynaptic glutamatergic neurotransmission in control and stressed mice for both the DBA/2J and C57BL strains. (Edited from Lowery-Gionta et al. (6))

3.2. Lesion or inactivation of the BLA prevents neuronal atrophy and reduces plasma corticosterone levels and glucocorticoid receptor response in the mPFC

The significance of lesion and/or inactivation studies is to assess whether a certain brain region of interest influences a process of interest. Consequently, measures on the process of interest are done at both the presence and the absence of the brain area. Measurements between 'presence' and 'absence' can then be compared to see if there is a significant difference in the measurements of the process of interest. Thus, the influence of a brain area of interest on a process of interest can be determined.

Up till now, several studies have demonstrated that lesion or inactivation of the basolateral amygdala (BLA) can prevent the atrophy observed in the mPFC (7, 45, 46). For instance, it has been demonstrated that lesion of the BLA prevents volume loss in the prelimbic area and anterior cingulate cortex in the mPFC following chronic stress. This volume loss was seen in superficial layers of the respective prefrontal areas. In addition, transient inactivation of the BLA, by using lidocaine that blocks sodium channels, produced the same effect (46).

Furthermore, some of the above-mentioned studies discussed the role of the BLA in the dysfunction and atrophy in the mPFC, regarding glucocorticoids. For example, impairments of working memory in the

mPFC following injections of corticosterone were prevented by lesions of the BLA (47). Given that an impaired working memory is correlated with a reduction in the volume of the mPFC (48), this is supportive for the idea that the BLA plays an important role in the stress-induced reduction of volume of the mPFC in relation to glucocorticoids. Moreover, permanent lesion and transient inactivation of the BLA with ibotenic acid and lidocaine, respectively, prevented the increase in expression of the glucocorticoid receptor in the prelimbic region (PL) and anterior cingulate cortex (ACC) of the mPFC (7) (see *figure 5*). This shows that the BLA can regulate the effect of glucocorticoids (indirectly) via regulating the number and density of the glucocorticoid receptors in this brain area (7). In addition to this, it is reported that lesion of the BLA significantly reduces the increase in plasma corticosterone levels that happens after chronic stress (49). This shows that an increase in plasma corticosterone is accompanied by an increase in glucocorticoid receptor expression in the mPFC and that this is regulated by the BLA. Together with the numerous studies that showed that chronic stress was accompanied by elevated corticosterone levels and substantial atrophy in the mPFC and that blocking glucocorticoid receptor function attenuated neuronal atrophy (8), it supports the idea that the BLA has a significant contribution in atrophy in the mPFC following chronic stress.

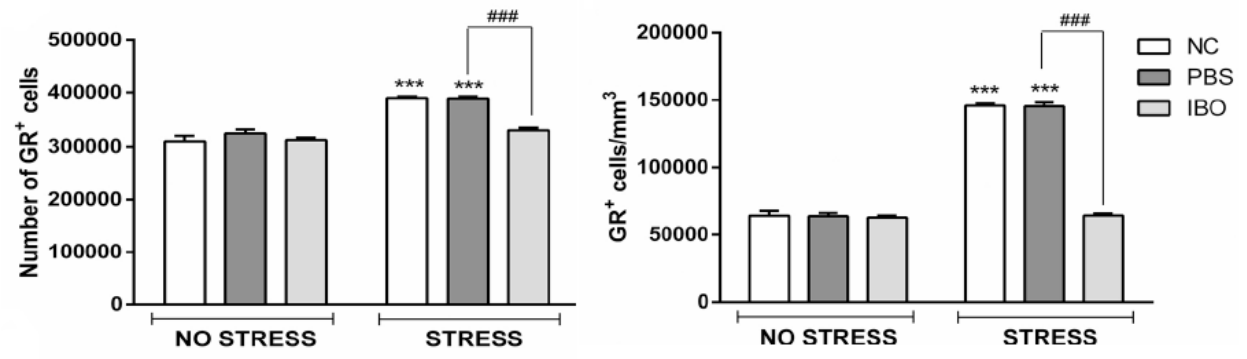

Figure 5. Glucocorticoid receptor number (left) and density (right) in the prelimbic area (PL). The following groups were used. Unstressed rats: naive, phosphate buffered saline infused and ibotenic acid (IBO) infused; stressed rats: idem. (Edited from Tripathi et al. (7))

Discussion

It has become clear that chronic stress has deleterious effects on structures in the prefrontal cortex and especially in the medial prefrontal cortex (mPFC). The major finding regarding structural alterations in the mPFC following stress, is the atrophy of dendrites and spines on the apical side of pyramidal neurons, mainly distal from the soma. Glutamatergic neurotransmission and glucocorticoids seem to play a major role in this, as was inferred from the studies done on structural properties of the mPFC following chronic stress, which was accompanied by a significantly elevated corticosterone concentration. Altogether, findings have come by from several perspectives that support the idea that the BLA-mPFC pathway plays an important role in the dendritic atrophy occurring in the mPFC.

Firstly, there is the altered glutamatergic neurotransmission onto pyramidal neurons in the mPFC. It is likely that part of this originates from the direct BLA projections, since anatomical studies revealed that

approximately 95% of these projections innervate the spines upon pyramidal neurons in the mPFC (27–29). It is also of note to mention that this includes spines on the apical dendrites (28) that are in turn affected by chronic stress. In addition, there were especially distally situated spines that were severed by chronic stress (4). These findings thus imply that elevated glutamatergic neurotransmission from the BLA may contribute to neuronal atrophy in the mPFC following chronic stress. On the other hand, a careful notion should be made here, since in the study of Lowery-Gionta et al. (6) it was seen that two strains had opposite alterations in their glutamatergic neurotransmission after a period of chronic stress. It might be that there is an innate component that determines alterations in glutamatergic output from the BLA. To pick up on this, an interesting question to pose is whether the mPFC is affected differently in its neuronal structure if there are opposite changes in glutamatergic output of neurons in the BLA. Also, one can combine the fact that neuronal atrophy occurs in multiple areas in the mPFC and that these same areas are innervated by the BLA. Although multiple studies show that the BLA innervates several areas in the mPFC that are severed after chronic stress, differences have been reported between areas in their extent to which they were innervated by the BLA (27). Furthermore, the extent to which neuronal atrophy takes place seems to differ between mPFC regions that are assessed (3, 13). This summons the question whether the density of glutamatergic innervation in an area in the mPFC correlates with the neuronal atrophy in the same area. If so, then this may be supportive for the role of glutamate in neuronal atrophy. In this case, anatomical procedures need to be combined with stress protocols to measure this. It might be wise to assess multiple layers in these studies, as studies on neuronal atrophy in the mPFC were accentuated on layer II/III of pyramidal neurons (2, 10, 12, 14).

Secondly, the BLA might also play a role in neuronal atrophy in the mPFC concerning its indirect projections towards the mPFC. Namely, besides the studies that indicated that PV interneurons are affected after chronic stress, there are also studies that demonstrate that activation of these neurons can prevent atrophy. Interestingly, pharmacogenetic activation of PV interneurons during stress prevented loss of spines on pyramidal neurons in the mouse barrel cortex (40). In a similar study, chemogenetic activation of PV interneurons counteracted the spine loss in the frontal association cortex following chronic stress (44). Together, the findings around PV interneurons imply that debilitation of PV interneurons during stress could lead to neuronal atrophy. However, as said before, it seems that studies on the effect of chronic stress on atrophy in relation to PV interneurons in the mPFC still need to be done, despite some recent findings on this in other brain areas. After all, it is also known that the BLA has glutamatergic connections with PV interneurons in the mPFC. Despite strain-dependent findings on glutamatergic neurotransmission from the BLA, it might be that an excessive glutamatergic neurotransmission from this region onto PV interneurons has excitotoxic effects as well that could contribute to the deterioration of these neurons. This in turn might lead to atrophy of pyramidal neurons in the mPFC. Nevertheless, the mechanisms behind the reversal of neuronal atrophy due to restoration of PV interneurons still need to be elucidated, as there may be a complex inhibitory circuitry in the mPFC consisting of multiple types of interneurons.

Lastly, it is reasonable to assume that the BLA-mPFC pathway is severed when the BLA is lesioned, since lesioning renders massive neuronal loss. This prevented the sensitivity of the mPFC to corticosterone, as it blocked the increase in glucocorticoid receptor (GR) densities in this area (7). Combining this with the observation that lesion of the BLA partially prevents a surge in corticosterone level following chronic stress (49), this indicates that not only GR levels are regulated by the BLA, but also glucocorticoids. Furthermore,

the increase of both the glucocorticoid levels and GR presence in the mPFC by the BLA suggest that the level of glucocorticoids as corticosterone is positively correlated with the presence of this receptor. Thus, the effect of corticosterone on the mPFC might be mainly explained by the activation of GRs herein. In particular, it is known that glucocorticoids play a crucial role in the hyperphosphorylation of the tau protein. On top of that, this effect was seen to be mediated by glucocorticoid receptors (9). In addition, tau hyperphosphorylation is closely associated with the degeneration of neuronal material (9). Lastly, atrophy of the mPFC was also prevented by lesion and inactivation of the BLA. Taking all this into consideration, an important role for the BLA in dendritic atrophy of the mPFC with regard to the effect of glucocorticoids and their receptors might exist.

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