The role of central amygdala neuronal types in drug-related and appetitive behaviors

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CHAPTER 6. GENERAL DISCUSSION

6.1 Conclusions

This thesis aimed to provide a better understanding about the role of different CeA neuronal types in appetitive and drug-related behaviors. Firstly, we showed that methamphetamine could robustly activate SST-expressing CeA neurons *in vivo* in males. This study is the first of its kind to do single-cell *in vivo* calcium imaging after a drug exposure, and it is a great start to ultimately be able to do single-cell *in vivo* calcium imaging while animals are self-administering drugs to understand the exact neuronal mechanisms underlying the development of addiction. In addition to the *in vivo* recordings, some alterations in synaptic activity of both Prkcd-expressing CeA neurons and SST-expressing CeA neurons in females were found, either acutely or 24 hours after methamphetamine exposure, respectively. These results showed us that methamphetamine alters the neuronal and synaptic activity of CeA neurons, and does so while differentially affecting males and females.

Secondly, we tried to examine the behavioral correlates of the neuronal and synaptic effects in the CeA after methamphetamine exposure. We examined several acute effects of methamphetamine, including methamphetamine-induced hyperactivity, the rewarding properties of methamphetamine, and the anxiogenic properties of methamphetamine. We manipulated SST-expressing, Prkcd-expressing and VIPR2-expressing CeA neurons while examining these behaviors, and found that these neuronal types do not play a role in these methamphetamine-related behaviors, in neither males nor females.

Thirdly, we looked at the role of VIPR2-expressing CeA neurons in homeostatic and appetitive behaviors. We found that inhibition of VIPR2-expressing CeA neurons in males specifically, could increase homeostatic feeding without altering body weight or locomotor activity. We did not see any effect from our manipulation in females. Interestingly, we examined the projections from VIPR2-expressing CeA neurons and found a strong projection to the lateral PBN. We hypothesize that this projection might play a role in the effects we see on feeding.

Lastly, we examined cAMP-induced AMPAR plasticity in the CeA, since this could be a possible mechanism through which drugs of abuse alter CeA activity. We set out to examine if CeA neurons were responsive to beta-adrenergic signaling stimulation. We found that high intracellular cAMP levels caused a robust potentiation of CeA neurons, which was independent of GluA3-containing AMPARs and likely also independent of GluA1-containing AMPARs.

6.2 Perspectives

This thesis provided more insight into the roles of the different neuronal CeA subtypes in various behaviors, but also introduced more questions. We have seen the robust neuronal activation and the synaptic alterations in SST-expressing CeA neurons and Prkcd-expressing CeA neurons, and hypothesized that these could underlie some acute effects from methamphetamine; the rewarding properties, the anxiogenic properties, or the methamphetamine-induced hyperactivity. However, we have demonstrated that neither SST-, Prkcd-, nor VIPR2-expressing CeA neurons were responsible for these behaviors. It is possible that the activation of CeA neurons from methamphetamine exposure is involved in other behavioral effects from methamphetamine. Psychostimulants are known to have varying acute effects on attention, concentration and cognitive ability in human and rodent subjects, with some inter-personal or inter-subject variability [137–141]. It is possible that CeA

neurons play some role in these behaviors. Additionally, there might be no direct behavioral correlates of these neuronal and synaptic CeA changes, and they are precursors for adaptations that could affect addiction susceptibility. Single drug exposures can already cause neural adaptations that lead to progressively more adaptations during subsequent drug use [75,142–144]. The role of the CeA in later stages of psychostimulant addiction has been a hot topic lately and has been well researched. It has been shown that the CeA is involved in a phenomena called "incubation of craving", in which the absence of a drug for addicted individuals will increase the craving for said drug, which consequently can lead to drug relapse [100,102–104,145,146]. It is possible that the synaptic and neuronal alterations that we see in the current study are a precursor for these behavioral effects. It would be interesting to follow the activity of these neurons across the development of addiction.

Apart from the observations about the role of the CeA in drug-related behaviors, this thesis also provided information about the role of VIPR2-expressing CeA neurons in feeding behaviors. Previous studies have found different roles in feeding for different neuronal CeA cell types, with Prkcd-expressing CeA neurons having negative feeding control, and Htr2a-expressing neurons having positive feeding control, amongst others [49,54]. In addition, it is known that infusion of PACAP into the CeA could inhibit feeding behaviors [114]. Since PACAP binds to VIPR2, it is possible that this peptide is involved in the effect we see from VIPR2 CeA inhibition on feeding. This thesis added another small piece of the puzzle by providing some indication that VIPR2-expressing CeA neurons are a part of the feeding circuit in the CeA, and more research needs to be done to specifically elucidate the mechanisms involved, including the role of CeA PACAP in feeding behavior.

Lastly, we examined AMPA-receptor plasticity in the CeA, as a possible mechanism underlying the effects of psychostimulants on the CeA. It is well known that psychostimulants increase extracellular monoamine concentrations, mainly dopamine, and to a lesser extent norepinephrine and serotonin [84]. When these monoamines bind to their receptors, intracellular cAMP levels are affected [147–149]. It has been shown in other brain areas, including the NAcc, that psychostimulant administration causes an increase in intracellular cAMP [150]. We have shown that a rise in intracellular cAMP levels upon beta-adrenergic stimulation in CeA neurons causes a robust synaptic potentiation. These are likely pathways through which psychostimulants can affect CeA synaptic activity also. It can be interesting to further elucidate the exact effects psychostimulants have on the synaptic activity and plasticity of CeA neurons.

6.3 Future directions

This research has been important to further our understanding of the role of different neuronal types in the CeA in various behaviors. However, more research needs to be done to further elucidate this. It has been mentioned before that the CeA has been implicated in late stages of psychostimulant addiction, including craving and relapse [102,145]. We have shown that different types of CeA neurons are affected by acute methamphetamine. Further research could focus on the question if we are looking at the same neurons that alter their activity and synaptic strength across the development of addiction, or if we are looking at different subsets of neurons. If the same neurons are involved, it could be of great importance to understand how exactly these neurons are altered, and which synaptic changes are happening across addiction development, to be able to target these changes for addiction treatment. Additionally, we have been focusing mainly on a psychostimulant, methamphetamine, but research has shown that the CeA might be involved in craving and relapse of different types of drugs [46,151–153]. This suggests that there are mechanisms at play in the CeA that are extrapolatable across different types of addiction,

making it all the more interesting to further elucidate what changes are happening in the CeA from acute first drug exposure to development of addiction.

Our results about the involvement of VIPR2-expressing CeA neurons in feeding behavior have a potential to be very interesting, but more research needs to be done to further understand the role of these neurons in feeding behavior. We have shown an effect from permanent VIPR2-expressing CeA inhibition in males, so a next step could be to use inducible and reversible inhibition methods such as DREADD or optogenetic manipulations to be able to compare within-subject effects. Additionally, it would be very interesting to see if optogenetic activation of VIPR2-expressing CeA neurons alone, or the VIPR2-expressing CeA -> lateral PBN pathway, could reduce feeding behaviors. Ultimately, recording the activity of these VIPR2-expressing CeA neurons through *in-vivo* calcium imaging will give us an understanding of the natural dynamics of these neurons during feeding behaviors. These are all very interesting avenues that need to be explored.

Overall, this thesis has given the field more insight into the role of different neuronal CeA cell types in various behaviors, but it is of great importance to further research these avenues.