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

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CHAPTER 2. METHAMPHETAMINE-INDUCED NEURONAL AND SYNAPTIC ACTIVITY IN THE CENTRAL AMYGDALA

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Abstract

Drug addiction remains a public health problem, and it is of importance to identify the neuronal and synaptic adaptations that are the underlying mechanisms leading to drug abuse. The central amygdala has been implicated in drug addiction, and in particular in psychostimulant use. We examined the effects of methamphetamine on synaptic and neuronal activity of CeA neurons. Through single-cell *in vivo* calcium imaging in the CeA, we show that methamphetamine can robustly activate a subset of SST-expressing CeA neurons in males. Surprisingly, we did not observe any effects from methamphetamine on synaptic activity of SST-expressing CeA neurons in males. However, methamphetamine caused synaptic depotentiation in SST-expressing and Prkcd-expressing CeA neurons in females, either 24 hours, or 30 min after methamphetamine exposure, respectively. These results show that there is a sex bias in the effects of methamphetamine on CeA neurons, which could lead to sex-differences in drug-related behaviors. The exact mechanisms underlying the effects we see is not known yet, and further research is necessary to elucidate the role of CeA neurons in drug-related behaviors.

2.1 Introduction

Drug addiction and drug-induced behaviors are thought to arise from neuronal and synaptic adaptations in circuits implicated in these behaviors. To be able to understand how changes in these circuits can contribute to the development of drug addiction, we have to first understand how certain drugs can alter the neuronal and synaptic activity of neuronal circuits. Synaptic activity and plasticity in several brain areas has been studied in relation to acute and repeated drug administration. Synaptic potentiation has been observed in the ventral tegmental area (VTA) and the nucleus accumbens (NAcc) after acute systemic cocaine and amphetamine administration^{74,75}. These synaptic adaptations caused by a single drug exposure could persist for at least 5 days, and these early changes are thought to be the base upon which subsequent drug use can ultimately lead to addiction. However, changes in CeA synaptic activity after acute psychostimulant use, and in particular methamphetamine use, have not been extensively studied.

We are the first to use *in vivo* single-cell calcium-imaging to assess real-life neuronal activity while animals are under the influence of methamphetamine. We show that methamphetamine causes robust changes in SST-expressing CeA neuron activities. Additionally, synaptic activity will be recorded *in vitro* after acute METH administration. Both measurements will be done immediately after METH administration, and 24 hours after, to be able to identify circuits involved in both the acute behavioral effects from METH, and to identify early changes that might be involved in the later development of addiction.

2.2 *In vivo* calcium imaging after methamphetamine administration

To assess the effect of methamphetamine on baseline CeA neuronal activity, we injected animals with methamphetamine and performed single-cell calcium imaging of SST-expressing CeA neurons. We used a cre-dependent GCaMP6 virus to selectively target SST-expressing neurons in SST-IRES-cre animals. One week after viral injection, animals were implanted with a GRIN lens above the CeA. The virus was allowed to express at least 5 weeks before starting imaging. Imaging comprised three days, with two imaging sessions per day, and was done in head-fixed animals. Animals were injected with either saline or methamphetamine (1 mg/kg, i.p.), 20 min before every imaging session. All three days started with a baseline saline session, followed by another saline session on day one and day three, and a methamphetamine session on day 2. Every session consisted of 30 minutes, with three minutes of imaging at the start, middle and end of the session, resulting in 9 minutes of imaging per session. Images were motion corrected. For every session, the STD (standard deviation) of GCaMP6 signals was calculated across the whole trace for every neuron that was imaged. A threshold of 2 times the STD was set, and the periods during which the GCaMP6 signals were above this threshold were considered as bouts of high neuronal activity⁷⁶. This analysis gives an indication of the significant fluctuations of neuronal activities away from average activities across time. When neuronal activity is compared between sessions, only neurons that can be tracked across all relevant sessions will be included.

2.2.1 Methamphetamine administration modulates the activity of SST-expressing CeA neurons

To assess the activity of SST-expressing CeA neurons after methamphetamine administration, SST-IRES-cre male animals were injected with a genetically-encoded calcium indicator GCaMP6 and a GRIN lens was implanted to image calcium signals, representing neuronal activity, after saline and methamphetamine injections. No significant differences were found between two saline sessions (SAL 1, SAL 2), indicating that baseline activity across saline sessions was stable across all neurons (Figure 2.1; paired t-test, $p > 0.05$), and averages across animals were similar, validating our analysis protocol.

When neuronal activity was compared between saline (SAL 2) and methamphetamine (METH) session, a significant increase was found across all neurons (Figure 2.1B; paired t-test, $p < 0.0001$), indicating that SST-expressing CeA neurons are robustly activated by methamphetamine administration. Similar effects were seen in average activity across animals. Neuronal activity 24 hours after methamphetamine administration (SAL 3) was significantly reduced compared to the methamphetamine session (Figure 2.1C; paired t-test, $p < 0.0001$), with similar effects seen across animals. Correspondingly, when activity of neurons tracked across all 3 days (SAL 2, METH and SAL3) was compared, a significant effect from session was found (Figure 2.D; one-way ANOVA, $p < 0.0001$), and post-hoc tests revealed significant differences between SAL 2 - METH session and METH - SAL 3 session (Tukey's multiple comparison test, $p = 0.0110$ and $p < 0.0001$, respectively), and no differences between SAL 2 - SAL 3 sessions (Tukey's multiple comparison test, $p > 0.05$). These results show that methamphetamine administration activated SST-expressing CeA neurons in males, and that activity of these neurons went back to baseline 24-hours after methamphetamine administration.

2.3 Synaptic activity after methamphetamine administration

Based on the results found during the *in vivo* imaging, we hypothesized that changes in synaptic strength could be responsible for the changes in SST-expressing CeA neuron activities. To examine this, we performed *in vitro* electrophysiology recordings of SST-expressing and Prkcd-expressing CeA neurons after methamphetamine

administration. SST-IRES-cre and Prkcd-2A-FlpO mouse lines were crossed with reporter lines to be able to identify neuronal types in slices. Animals were injected with either saline or methamphetamine (1 mg/kg, i.p.) either 30 minutes or 24 hours before making slices, and miniature excitatory postsynaptic currents (mEPSCs) were recorded from SST-expressing and Prkcd-expressing neurons in the CeA.

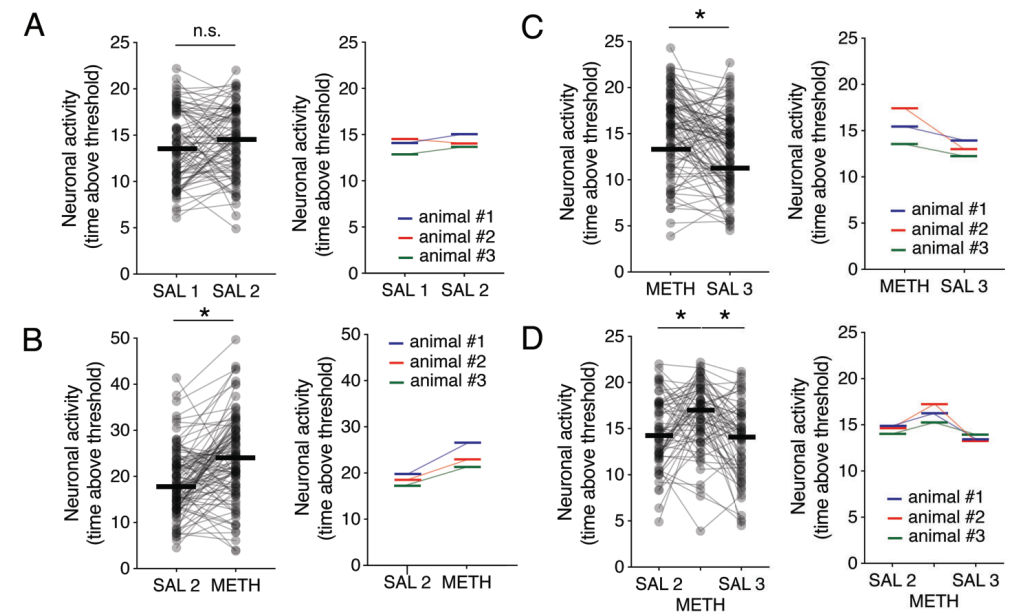


Figure 2.1 Neuronal activity of SST-expressing CeA neurons in males after methamphetamine administration. (A) No difference in neuronal activity was found between saline sessions across all neurons (paired t-test, $p > 0.05$), and averages across animals were similar. (B) Neuronal activity was significantly higher during METH session compared to saline session across all neurons (paired t-test, $p < 0.0001$), and averages across animals showed a similar trend. (C) Neuronal activity was decreased 24 hours after METH administration (paired t-test, $p < 0.0001$), and similar trends were seen across animals. (D) Neuronal activity during saline, meth, and saline sessions was increased after METH, and activity 24 hours after METH administration went back to baseline (SAL 2) (one-way ANOVA, $p < 0.0001$). Data represented as mean \pm s.e.m.

2.3.1 Synaptic activity of SST-expressing CeA neurons after methamphetamine administration

SST-reporter line animals were injected with either saline or methamphetamine, and slices were made 30 minutes after injection to examine changes in synaptic activity while animals are under the influence of methamphetamine. Due to the literature showing differences between sexes in drug-related behaviors, all data was separated based on sex⁵⁶. Surprisingly, no differences were found between mEPSC frequency or amplitude of neurons from females injected with saline or methamphetamine (Figure 2.2A; unpaired t-test, $p > 0.05$). In addition, neurons from males injected with saline or methamphetamine showed similar mEPSC frequency and amplitude as well (Figure 2.2C; unpaired t-test, $p > 0.05$). These results demonstrate that there is no effect from methamphetamine on mEPSCs recorded from SST-expressing neurons, 30 minutes after administration.

Even though no effects were found from methamphetamine treatment on mEPSCs recorded from SST-expressing CeA neurons at 30 min, we wanted to examine if methamphetamine treatment had any lasting effects on synaptic activity 24 hours after the injection. A synaptic depression effect from methamphetamine was found in SST-expressing neurons 24 hours after administration, in females only. mEPSC frequency and amplitude was significantly reduced in neurons from females 24 hours after methamphetamine administration, compared to saline administration (Figure 2.3A; unpaired t-test, $p=0.0342$). This effect was not found in males; in which no differences were seen in mEPSC frequency or amplitude 24 hours after methamphetamine or saline administration (Figure 2.3C; unpaired t-test, $p>0.05$). This shows that methamphetamine can induce a depression of excitatory synapses 24 hours after administration in SST-expressing CeA neurons in females only.

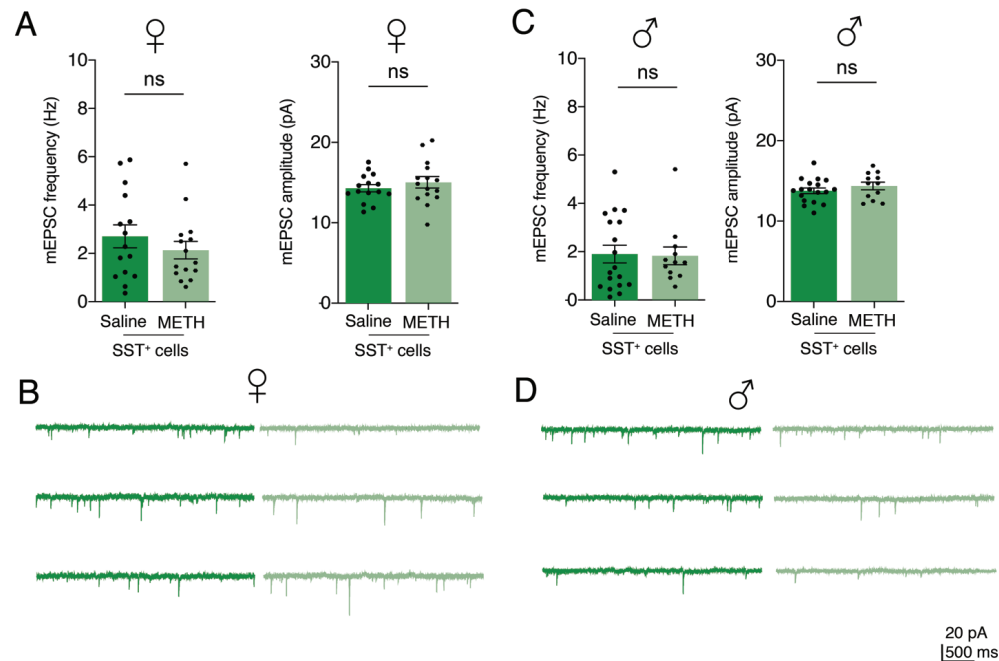


Figure 2.2 Synaptic activity of SST-expressing CeA neurons 30 minutes after methamphetamine administration. (A) No significant differences were found in mEPSC frequency or amplitude of SST-expressing neurons from females injected with either methamphetamine (METH) or saline (unpaired t-test, $p>0.05$). (B) Example traces from neurons shown in panel (A). (C) No significant differences were found in mEPSC frequency or amplitude of SST-expressing neurons from males injected with either methamphetamine (METH) or saline (unpaired t-test, $p>0.05$). (D) Example traces from neurons shown in panel (C). Data represented as mean \pm s.e.m.

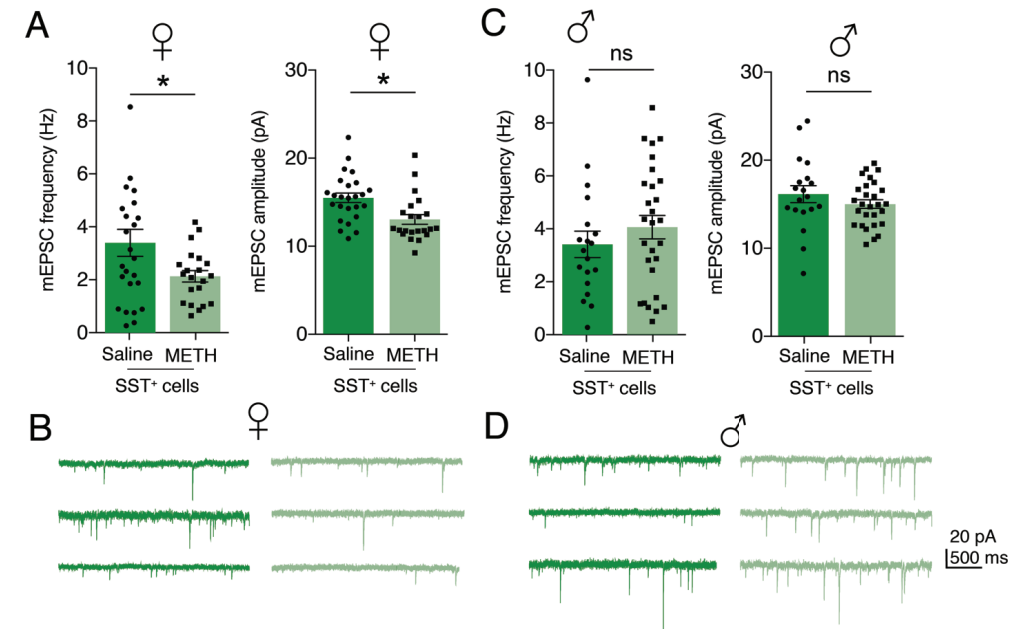


Figure 2.3 Synaptic activity of SST-expressing CeA neurons 24 hours after methamphetamine administration. (A) Both mEPSC frequency and amplitude of SST+ neurons from female animals injected with methamphetamine (METH) was significantly lower compared to neurons from females injected with saline (unpaired t-test, $p=0.0342$, $p=0.0040$, respectively). (B) Example traces from neurons shown in panel (A). (C) No significant differences were found in mEPSC frequency or amplitude of SST+ neurons from males injected with either methamphetamine (METH) or saline (unpaired t-test, $p>0.05$). (D) Example traces from neurons shown in panel (C). Data represented as mean \pm s.e.m.

2.3.2 Synaptic activity of Prkcd-expressing CeA neurons after methamphetamine administration

To assess if the effects from methamphetamine we see on SST-expressing CeA neurons in females is specific to SST-expressing neurons, *prkcd*-reporter line animals were injected with either saline or methamphetamine, and slices were made 30 minutes after injection to examine changes in synaptic activity while animals are under the influence of methamphetamine. Data were separated based on sex.

A reduction in mEPSC amplitude was observed in *Prkcd*-expressing neurons from females 30 min after methamphetamine administration (Figure 2.4A; unpaired t-test, $p=0.0023$). No difference was seen on mEPSC frequency (Figure 2.4A; unpaired t-test, $p>0.05$). In addition, no differences were found in mEPSC frequency or mEPSC amplitude of neurons from males injected with saline or methamphetamine (Figure 2.4C; unpaired t-test, $p>0.05$). These results demonstrate that there is a small effect of methamphetamine on synaptic activity of *Prkcd*-expressing neurons in females, 30 minutes after administration.

We did not find any effect from methamphetamine on *Prkcd*-expressing synaptic activity 24 hours after methamphetamine administration. mEPSC frequency and amplitude of *Prkcd*-expressing neurons from females did not differ between saline and methamphetamine injected animals (Figure 2.5A; unpaired t-test, $p>0.05$). In addition, no effects from methamphetamine on *Prkcd*-expressing synaptic activity were seen in males injected with methamphetamine (Figure 2.5C; unpaired t-test, $p>0.05$).

These results show that methamphetamine has an effect on Prkcd-expressing neurons 30 min after methamphetamine administration, and this effect is only seen in females.

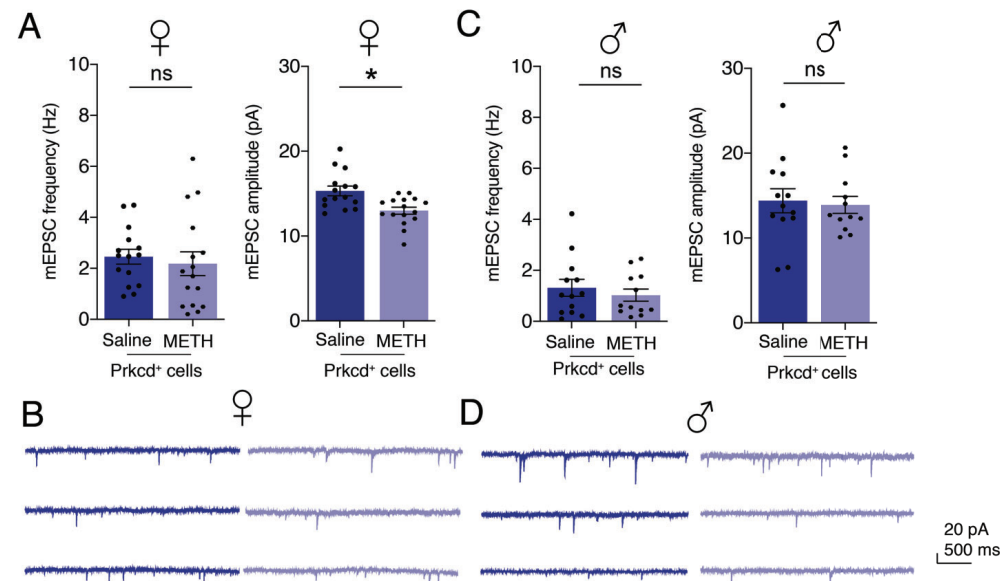


Figure 2.4 Synaptic activity of Prkcd-expressing CeA neurons 30 minutes after methamphetamine administration. (A) No significant differences were found in mEPSC frequency or amplitude of Prkcd+ neurons from females injected with either methamphetamine (METH) or saline (unpaired t-test, $p > 0.05$). (B) Example traces from neurons shown in panel (A). (C) No significant differences were found in mEPSC frequency or amplitude of Prkcd+ neurons from males injected with either methamphetamine (METH) or saline (unpaired t-test, $p > 0.05$). (D) Example traces from neurons shown in panel (C). Data represented as mean \pm s.e.m.

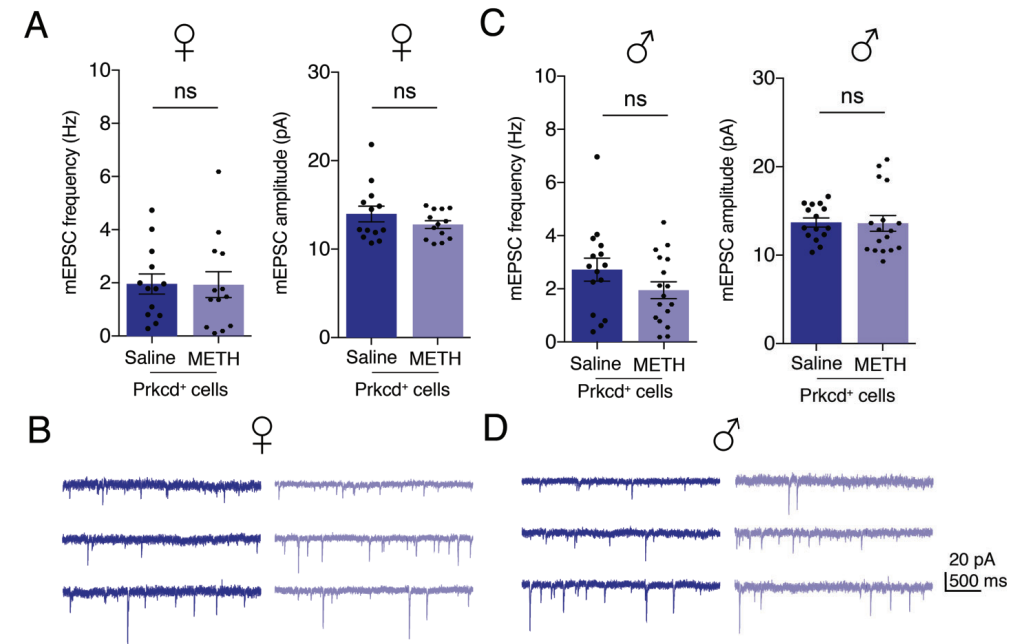


Figure 2.5 Synaptic activity of Prkcd-expressing CeA neurons 24 hours after methamphetamine administration. (A) No significant differences were found in mEPSC frequency or amplitude of Prkcd+ neurons from females injected with either methamphetamine (METH) or saline (unpaired t-test, $p > 0.05$). (B) Example traces from neurons shown in panel (A). (C) No significant differences were found in mEPSC frequency or amplitude of Prkcd+ neurons from males injected with either methamphetamine (METH) or saline (unpaired t-test, $p > 0.05$). (D) Example traces from neurons shown in panel (C). Data represented as mean \pm s.e.m.

2.4 Discussion

In this study, we examined the effects of methamphetamine on synaptic and neuronal activity of CeA neurons. As a first in our field, we have done single-cell *in vivo* calcium imaging in the CeA while animals are under the influence of a drug. We showed that a single methamphetamine dose could robustly activate a subset of SST-expressing CeA neurons *in vivo* in males, and the activity of these neurons went back to baseline 24 hours after methamphetamine exposure. Possibly, methamphetamine administration caused an increase in neuronal firing through increased synaptic input, or an increase in intrinsic excitability. Naturally, these recordings need to be repeated in females, and it would be interesting to investigate the activity of other neuronal types. However, the question remains what the behavioral correlate of this neuronal activity is. Several studies have shown that activation of the CeA as a whole, could intensify the motivation to pursue a reward, including drug rewards such as cocaine^{14,44,77}. In addition, some older studies using both intracranial CeA delivery or self-administration of psychostimulants, the type of drug that methamphetamine falls under, point towards a role of the CeA in the rewarding properties of psychostimulants^{78,79}. Possibly, the SST-expressing neurons are responsible for some rewarding properties of methamphetamine, and this is characterized by the robust increase in neuronal activity. Further research is necessary to examine this.

In addition to the *in vivo* imaging, we also demonstrated that methamphetamine could induce changes in

synaptic transmission in SST-expressing CeA neurons, 24 hours after methamphetamine exposure. Surprisingly, this effect was only observed in females and we did not see any alterations in synapse strength in either cell type in males. It is hard to compare *in vivo* imaging with *in vitro* electrophysiology, since we are comparing action potential-dependent responses with synaptic transmission, two different scales, which do not necessarily translate to each other. In addition, we only have *in vivo* imaging for males, and we are left to hypothesize how SST-expressing CeA neuronal activity is affected by methamphetamine in females. However, we know that females can respond differently to drugs of abuse, and in particular the effects of female gonadal hormones, like estrogen, have been documented to affect dopamine signaling^{55,57-59}. It is possible that SST-expressing CeA neuronal activity in females is also increased after methamphetamine administration, possibly even more than males. In addition, the heightened response of females to dopamine release after drug administration, might introduce compensatory mechanisms that caused a down regulation of certain postsynaptic receptors, leading to the reduction in synaptic activity 24 hours after drug exposure.

Additionally, we saw methamphetamine could cause a reduction in mEPSC amplitude of Prkcd-expressing neurons, only in females as well. Since we do not have information on *in vivo* activity of Prkcd-expressing CeA neurons after methamphetamine administration, we are left to hypothesize about the role of this reduced synaptic activity. It is possible methamphetamine directly affects the postsynaptic receptors of CeA Prkcd-expressing neurons in females. Again, the sex differences in response to drugs of abuse can explain the difference in Prkcd-expressing synaptic activity between males and females.

In addition to specific effects from methamphetamine on SST-expressing or Prkcd-expressing CeA neurons, it is also possible that methamphetamine affects inhibitory synaptic inputs onto these cells, thereby causing a change in synaptic activity of SST- and Prkcd-expressing CeA neurons. Furthermore, SST-expressing and Prkcd-expressing neurons have reciprocal connections between and within themselves, which could also be affected by methamphetamine administration.

These results have shown us that there is still a lot to unpack about the effects of methamphetamine, and psychostimulants in general, on CeA neurons. It is very clear that there is a sex bias in the effects of this drug on CeA neurons, which could lead to sex-differences in behavior, and it is of importance to further examine these differences to be able to hopefully produce tailored treatments to prevent and treat drug addiction.