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

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CHAPTER 3. CHARACTERIZING THE ROLE OF NEURONAL TYPES IN THE CENTRAL AMYGDALA IN ACUTE METHAMPHETAMINE-INDUCED BEHAVIORS

Anna Lien Bouhuis, Bo Li

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Abstract

Methamphetamine is a psychostimulant drug that produces euphoria and induces behavioral activation through increase in locomotion. Due to its highly addictive nature, recreational methamphetamine use can quickly escalate to chronic methamphetamine abuse. Despite years of research on the mechanisms underlying methamphetamine addiction, there is no pharmacological treatment available and patients have to resort to solely behavioral therapies. To be able to identify appropriate pharmacological therapy targets, it is of importance to understand the acute and chronic effects methamphetamine has on the brain and how these effects can be translated to drug-related behaviors. Several studies have demonstrated a role for the central amygdala in different stages of methamphetamine use. However, no neuronal types have been identified within the central amygdala concerning methamphetamine abuse. Using cell-type specific inhibitions, we examine the role of three cell populations in the central amygdala, Somatostatin (SST), Prkc-delta (Prkcd) and VIPR2 in the rewarding and anxiogenic properties of methamphetamine. Inhibition of these neuronal types did not affect the rewarding effects of methamphetamine in neither males nor females, suggesting that these neurons do not mediate these behaviors in either sexes. Additionally, we saw a robust increase in anxiety behaviors after methamphetamine administration, but were unable to alter this after inhibition of the different neuronal types. These results suggest that the CeA is not significantly involved in either the rewarding, or the anxiogenic properties of methamphetamine.

3.1 Introduction

Methamphetamine use and abuse in the United States and across the world remains a significant health problem⁸⁰. Methamphetamine belongs to a group of drugs that are called psychostimulants, which includes amphetamine and cocaine as well. Psychostimulants cause behavioral activation and can increase attention and concentration. Long-term psychostimulant use can have devastating effects, including weight loss, paranoia and death⁸⁰⁻⁸². Psychostimulants exert their effects through an increase in monoamines, such as dopamine, norepinephrine and serotonin, in several brain areas⁸³⁻⁸⁵. While some pharmacological treatments are available for other classes of drug addictions, no food and drug administration (FDA)-approved treatment is currently available for psychostimulant, and in particular methamphetamine, addiction. To be able to develop appropriate treatments, it is of importance to understand the acute and chronic effects methamphetamine has on the brain and how these effects might mediate methamphetamine-related behaviors.

Several studies have implicated the central amygdala (CeA) in rewarding and addictive behaviors^{14,17,41,42,44}. The CeA is a sub nucleus of the extended part of the amygdala, which consists of a lateral (CeL) and medial (CeM) part and the vast majority of neurons in this area are GABA-ergic neurons⁸⁶. Multiple neuronal types have been identified in the CeA, with the two major cell types being Prkcd-expressing and somatostatin (SST)-expressing neurons^{9,87}. SST- and Prkcd-expressing neurons have been implicated in several different behaviors, including appetitive and aversive behaviors^{17,28}. In addition, preliminary data from our lab show us a newly identified CeA neuronal type: VIPR2-expressing neurons. They have some overlap with SST-expressing neurons

and to a lesser extent with Prkcd-expressing neurons, and its role in behavior has not been studied yet.

The CeA has been characterized as a centralizing area in which emotionally salient stimuli are processed into appropriate physiological and behavioral responses. In addition to its role in fear processing^{7,9,16}. The CeA has also been implicated in appetitive behaviors, such as reward processing and reward seeking, food consumption, and drug-related behaviors^{14,17,41,42,44,49}. The CeA has been shown to be involved in several stages of drug use as well as different drug classes. Early studies have used intracranial drug delivery to pinpoint brain areas that might be involved in the rewarding properties of a certain drug. Direct intracranial delivery of amphetamine into the CeA could robustly produce drug-induced conditioned place preference and an increase in locomotion⁷⁸. In addition, rats would intracranially self-administer amphetamine into the CeA, indicating that the CeA might be involved in mediating rewarding properties of amphetamine⁷⁹. Furthermore, the reinforcing properties of cocaine self-administration in rats were reduced when dopamine receptor antagonists are directly infused into the CeA⁸⁸. These studies together could indicate that the CeA could have a role in mediating behavioral and rewarding effects of several psychostimulants. Correspondingly, CeA neurons show an increase in fos-B expression after acute and repeated methamphetamine self-administration, demonstrating an activation of the CeA after methamphetamine use⁸⁹, which further indicates that the CeA could have a role in mediating behavioral and rewarding effects of several psychostimulants. In contrast, one study has shown that lesions of the CeA did not affect either the acquisition or the expression of amphetamine-induced conditioned place preference⁹⁰, arguing against a role for CeA in mediating the rewarding properties of psychostimulants. This shows that there is some ambiguity about the role of the CeA in the rewarding properties of psychostimulants.

Psychostimulants can, apart from the rewarding effects, have an influence on anxiety levels as well; with most studies showing an increase in anxiety from an acute dose of amphetamine or methamphetamine⁹¹⁻⁹³. Most of these studies are done in males, even though it is known that drug use and development of abuse can differ greatly between males and females⁵⁶. Several studies have also implicated the central amygdala in anxiety behaviors^{36,94,95}, which could also implicate the central amygdala in anxiety behavior after psychostimulant use.

Most of the above-mentioned studies have focused on cocaine and amphetamine, and very few on methamphetamine. While cocaine and amphetamine increase monoamine levels in the brain similar to methamphetamine, the exact mechanism and potency by which they do so differ⁸⁴. Cocaine mainly blocks monoamine reuptake inhibitors, while amphetamine and methamphetamine affect both monoamine reuptake inhibitors, as well as increase the release of monoamines^{96,97}. In addition, the precise role of the CeA, and in particular the different neuronal types within the CeA, in acute methamphetamine behaviors has not been determined. Therefore, in this study, we focus on the rewarding properties of methamphetamine and its effects on anxiety behaviors, and we try to disentangle the role of the different neuronal types within the CeA in these behaviors.

3.2 Methamphetamine-induced conditioned place preference and hyperactivity

To assess the role of different CeA neuronal types in the rewarding properties of methamphetamine, we ran animals on a methamphetamine-induced conditioned place preference task while selectively inhibiting activity of specific CeA neuronal subtypes. In the drug-induced conditioning place preference paradigm, a previously unpreferred context will be repeatedly paired with a drug exposure, causing the animal to associate the rewarding methamphetamine experience with this context and switching its preference. During testing day, time spent in the previously non-preferred context will be a measurement of how rewarding methamphetamine is for an animal.

We used a cre-dependent virus expressing tetanus toxin-light chain (TeLC), which blocks neurotransmitter release, to permanently inhibit neurons in which the virus is expressed⁹⁸. Control animals were injected with a cre-dependent GFP virus. We injected this virus into the CeA of either SST-IRES-cre, Prkcd-IRES-cre or VIPR2-IRES-cre mice to inhibit SST-expressing, Prkcd-expressing or VIPR2-expressing CeA neurons, respectively. The ability of the cre-dependent TeLC virus to affect CeA neurons has been demonstrated before through effects on fear learning⁹⁹. Animals were then trained on the methamphetamine-induced conditioned place preference paradigm, and methamphetamine-induced hyperactivity (on conditioning days) and the rewarding properties of methamphetamine (on post-test day) were assessed (Figure 3.1).

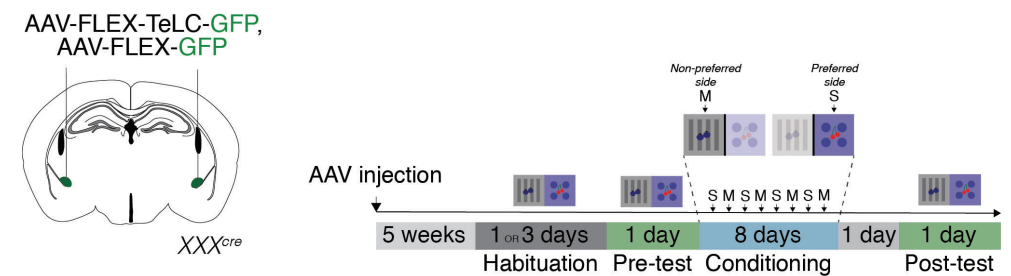


Figure 3.1 Methamphetamine-induced conditioned place preference protocol

3.2.1 The role of SST-expressing CeA neurons in methamphetamine-induced conditioned place preference and hyperactivity

To assess the role of SST-expressing CeA neurons in the hyperactivity and rewarding properties of methamphetamine, SST-cre animals were injected in the CeA with cre-dependent TeLC or cre-dependent GFP (figure 3.2A,B). No significant differences were found between GFP and TeLC animals on velocity during habituation day, suggesting no effect from inhibition of SST-expressing CeA neurons on baseline locomotor activity (Figure 3.3A; unpaired t-test, $p > 0.05$). During conditioning, velocity was measured and compared between saline and corresponding methamphetamine sessions, to assess the effect of methamphetamine on velocity. No significant differences were found between GFP and TeLC animals in hyperactivity induced by methamphetamine in either females or males (Figure 3.3B; Two-way ANOVA, $p > 0.05$). There was a significant effect of sessions on velocity in females (Figure 3.3B; Two-way ANOVA effect of session, $p < 0.0001$), with post-hoc tests revealing significance when comparing velocity during saline session vs corresponding methamphetamine sessions predominantly in the later sessions; S2 vs M2 (GFP - $p = 0.0321$, TeLC - $p < 0.0001$), S3 vs M3 (GFP - $p > 0.05$, TeLC - $p < 0.0001$) and S4 vs M4 (GFP - $p = 0.0059$, TeLC - $p < 0.0001$) are mostly significantly different in both GFP and TeLC females (Sidak's multiple comparison tests). Similarly, in males there was also a significant effect of sessions on velocity (Figure 3B; Two-way ANOVA effect of session, $p < 0.0001$), with post-hoc tests revealing significance primarily when later sessions are compared; S2 vs M2 (GFP - $p = 0.0247$, TeLC - $p > 0.05$), S3 vs M3 (GFP - $p = 0.0309$, TeLC - $p = 0.0210$) and S4 vs M4 (GFP - $p = 0.0077$, TeLC - $p = 0.0082$) are mostly significantly different in both GFP and TeLC males (Sidak's multiple comparison tests). These results indicate methamphetamine-induced hyperlocomotion in later sessions (i.e. S4 vs M4), while showing no effect from methamphetamine on locomotion in earlier sessions (i.e. S1 vs M1). These results are in concordance with literature describing locomotor sensitization of psychostimulants with multiple drug exposures, demonstrated by an increase in drug-induced

hyper locomotion with subsequent drug exposures.

After conditioning, animals were recorded during testing day and time spent in methamphetamine-paired chamber during pre- and posttest was used to calculate a subtracted CPP score ((posttest-pretest)/pretest) and CPP score (posttest-pretest) per animal. No difference was found between GFP and TeLC animals on subtracted CPP score or CPP score, in females or males (Figure 3.3C; unpaired t-tests, all $p > 0.05$). In addition, time spent in methamphetamine-paired chamber was compared pre- and posttest for all groups (Figure 3.3D). No significant differences were found between GFP and TeLC animals in neither females nor males (Two-way ANOVA, all $p > 0.05$). However, both females and males show a significant effect of session (females – $p = 0.0002$, males – $p = 0.0178$), demonstrating an increase in time spent posttest vs pretest in all groups. These results together show that methamphetamine administration could effectively increase time spent in a previously un-preferred chamber, suggesting that methamphetamine was rewarding for all groups, and CeA SST inhibition did not affect this.

As shown above, permanent inhibition of CeA SST-expressing neurons did not alter methamphetamine-induced hyperlocomotion, locomotor sensitization, or rewarding properties of methamphetamine, in neither females nor males.

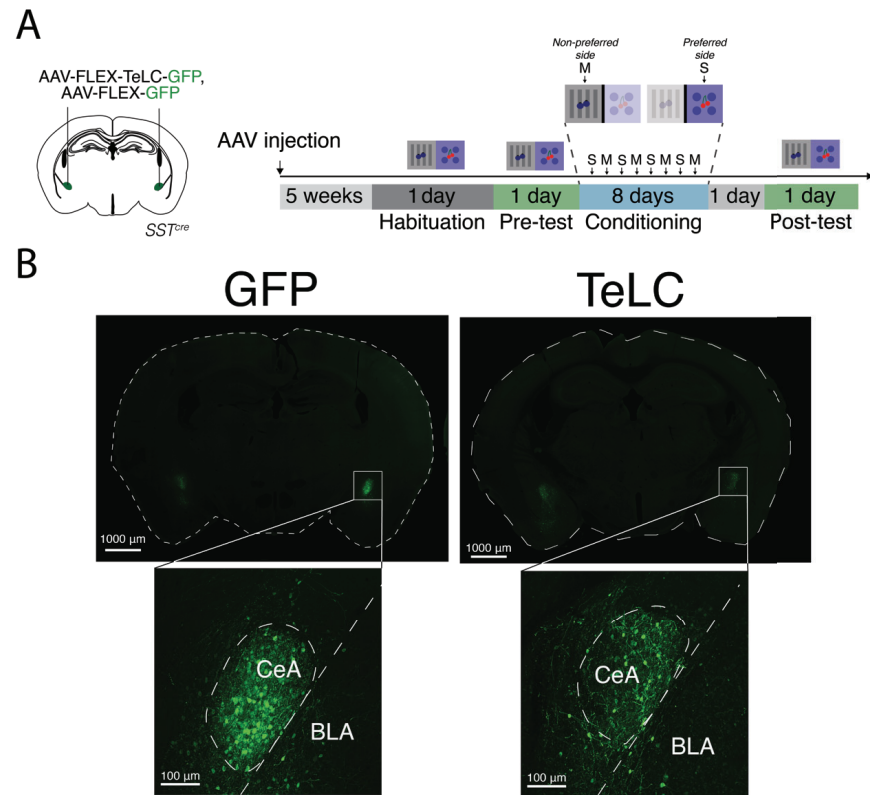


Figure 3.2. Expression of TeLC and GFP virus in SST-expressing CeA neurons. (A) Schematic of experimental methods. (B) Representative histology images of a GFP (left) and TeLC (right) injected animal

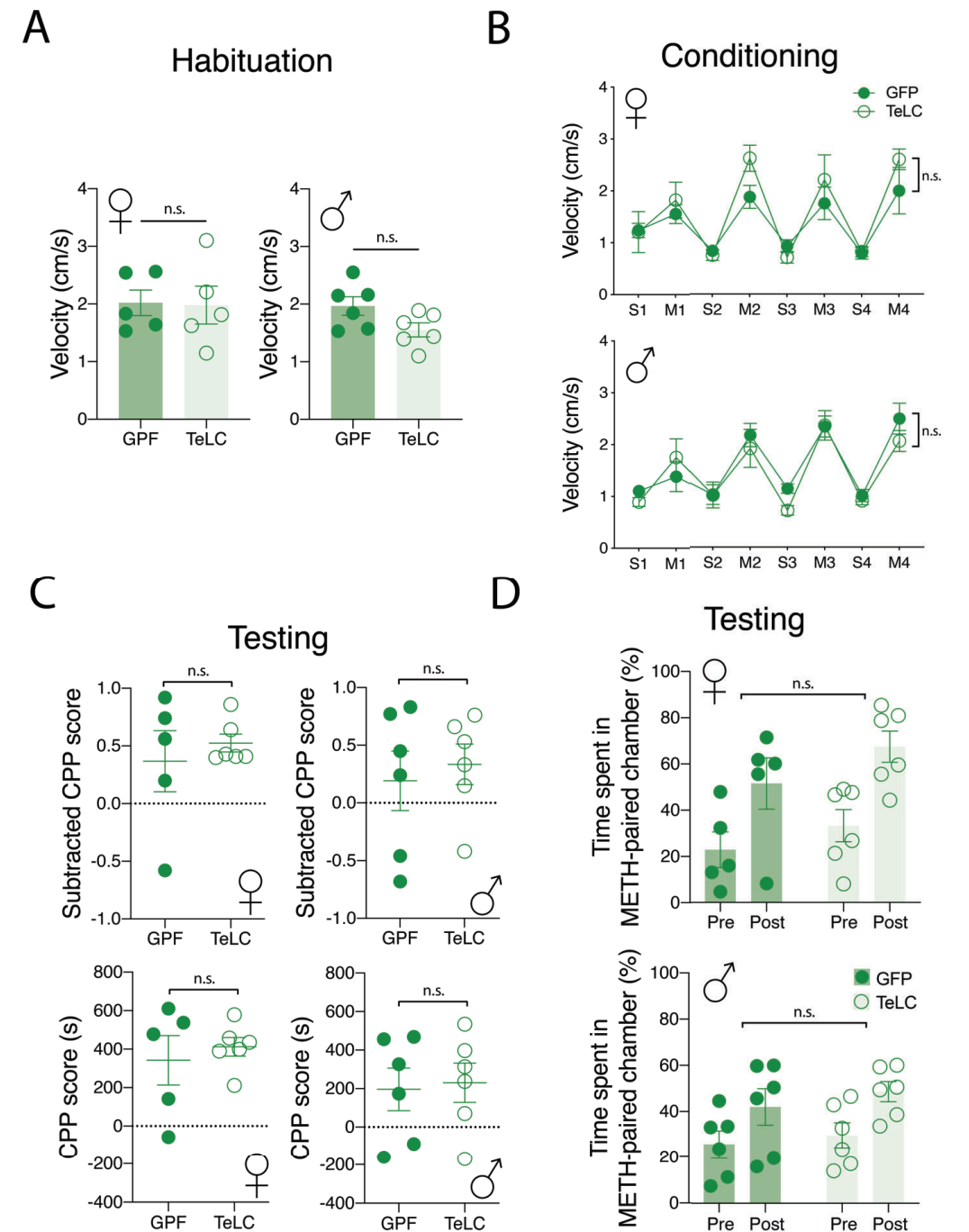


Figure 3.3 (previous page) SST-expressing CeA neurons do not play a role in methamphetamine-induced hyperactivity or methamphetamine-induced conditioned place preference. (A) No difference in velocity during habituation sessions in GFP and TeLC females (unpaired t-test, $p>0.05$) or GFP and TeLC males (unpaired t-test, $p>0.05$). (B) No difference has been found on saline (S) or methamphetamine (M) sessions in GFP and TeLC females (two-way ANOVA, $p>0.05$) or GFP and TeLC males (two-way ANOVA, $p>0.05$). A significant effect of session is found for both males and females (two-way ANOVA, all $p<0.0001$). (C) On testing day, neither female nor male GFP and TeLC animals show any difference on subtracted CPP score (Females - unpaired t-test, $p>0.05$; Males - unpaired t-test, $p>0.05$), or CPP score (Females - unpaired t-test, $p>0.05$; Males - unpaired t-test, $p>0.05$). (D) No difference is observed between female GFP and TeLC animals on time spent in METH-paired chamber pretest (Pre) and posttest (Post) (two-way ANOVA, $p>0.05$), but a significant effect from session has been found (two-way ANOVA, $p=0.0002$). Similarly, no difference has been shown between male GFP and TeLC animals on time spent in METH-paired chamber pre- and posttest (two-way ANOVA, $p>0.05$), but a significant effect of session is shown (two-way ANOVA, $p=0.0178$). Data represented as mean \pm s.e.m.

3.2.2 The role of Prkcd-expressing CeA neurons in methamphetamine-induced conditioned place preference and hyperactivity

To assess the role of Prkcd-expressing CeA neurons in the hyperactive and rewarding properties of methamphetamine, Prkcd-IRES-cre animals were injected in the CeA with cre-dependent TeLC or GFP (Figure 3.4A,B). No differences in velocity were found during habituation session between GFP and TeLC animals, in either females or males (Figure 3.5A; unpaired-test, all $p>0.05$), demonstrating no effect from Prkcd-expressing CeA inhibition on baseline locomotor activity. No differences were found between GFP and TeLC animals in hyperactivity induced by methamphetamine in either females or males (Figure 3.5B; two-way ANOVA, all $p>0.05$). However, a significant effect of session was found for females ($p<0.0001$), with post-hoc test revealing mostly significant differences between S1 vs M1 (Sidak's multiple comparison tests; GFP - $p>0.05$, TeLC - $p=0.0026$), S2 vs M2 (GFP - $p=0.0007$, TeLC - $p<0.0001$), S3 vs M3 (GFP - $p<0.0001$, TeLC $p<0.0001$) and S4 vs M4 (GFP - $p<0.0001$, TeLC - $p<0.0001$).

Similar results were found in males, with all saline sessions having significantly lower velocity than methamphetamine sessions; S1 vs M1 (GFP - $p=0.0002$, TeLC - $p=0.0224$), S2 vs M2 (GFP - $p<0.0001$, TeLC - $p<0.0001$), S3 vs M3 (GFP - $p<0.0001$, TeLC - $p<0.0001$) and S4 vs M4 (GFP - $p<0.0001$, TeLC - $p=0.0032$). These results show that methamphetamine induced robust hyper locomotion in both GFP and TeLC groups, for females and males. Since most groups already show a significant increase in velocity during their first methamphetamine session, no definitive conclusion can be made about the effect of our manipulation on locomotor sensitization. However, no differences are found between GFP and TeLC groups on velocity, therefore it is likely that there is no difference in locomotor sensitization. No effect was found from Prkcd-expressing neuronal inhibition on the rewarding properties of methamphetamine in either males or females (Figure 3.5C; unpaired t-tests, all $p>0.05$). In addition, no differences were found between GFP and TeLC animals on time spent in methamphetamine-paired chamber pre- and posttest (two-way ANOVA, females - $p<0.0001$, males - $p<0.0001$), while a significant effect of session was found in all groups (all $p<0.0001$). These findings show that methamphetamine could robustly increase time spent in the previously un-preferred context after pairing with methamphetamine, suggesting a rewarding effect of methamphetamine in all groups. To summarize, Prkcd-expressing neurons are not involved in mediating methamphetamine induced hyper-locomotion, nor are they involved in regulating the rewarding effects of methamphetamine.

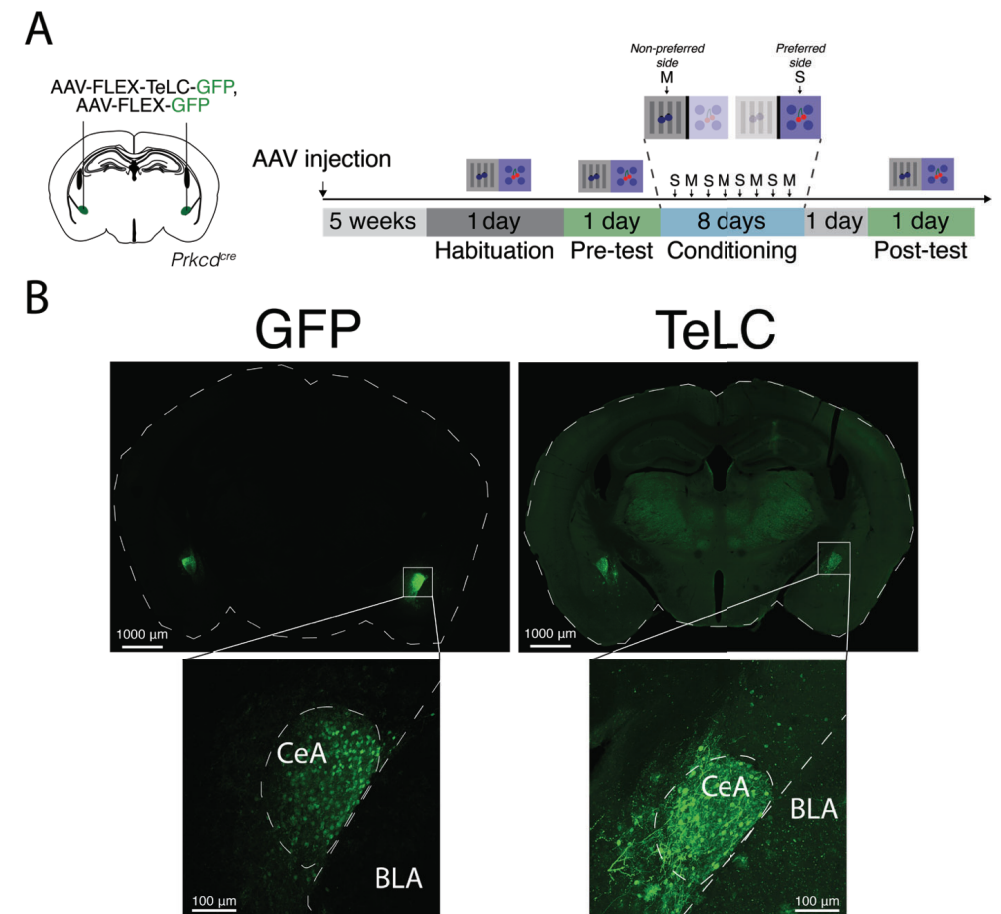


Figure 3.4. Expression of TeLC and GFP virus in Prkcd-expressing CeA neurons. (A) Schematic of experimental methods. (B) Representative histology images of a GFP (left) and TeLC (right) injected animal.

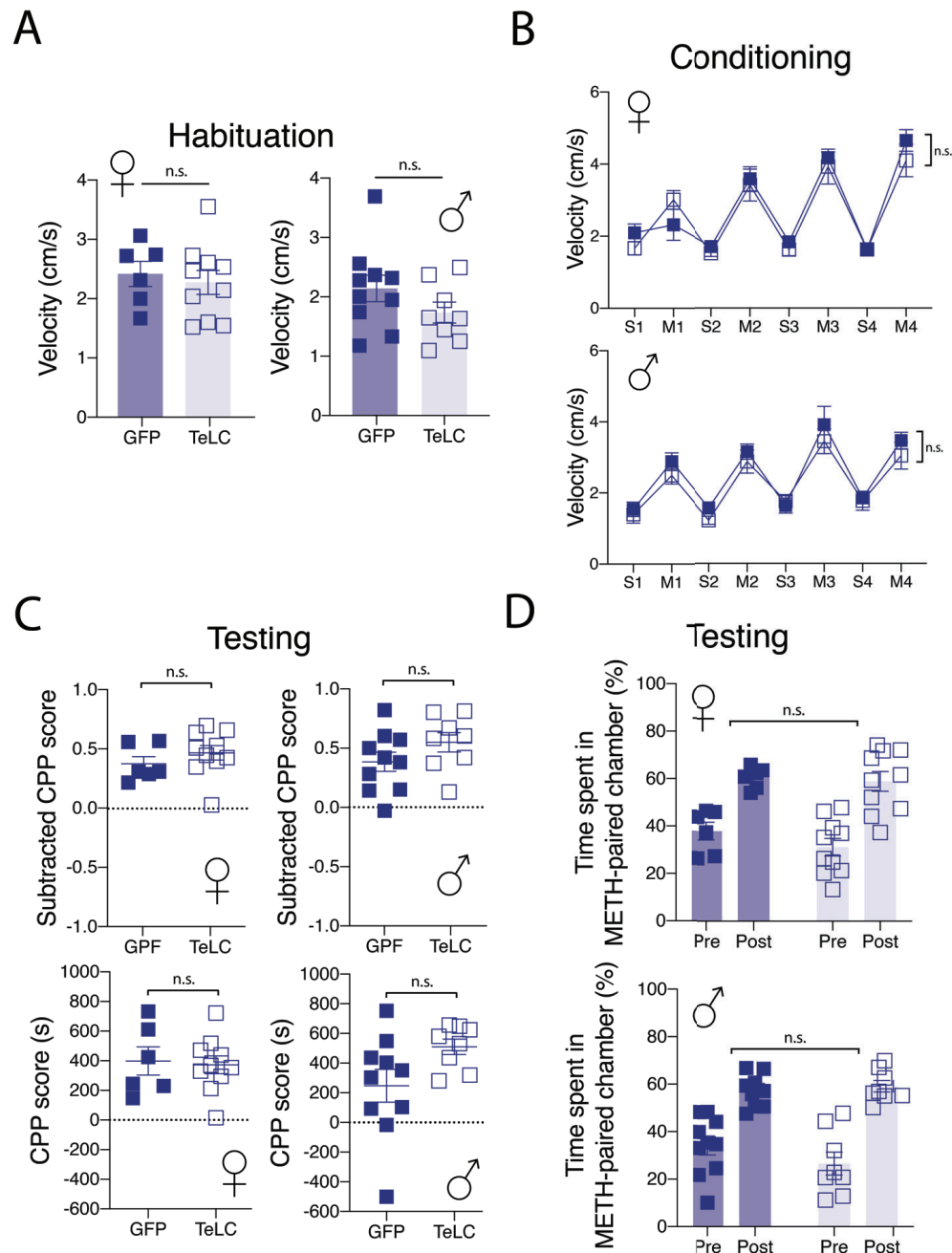


Figure 3.5 (previous page) Prkcd-expressing CeA neurons do not play a role in methamphetamine-induced hyperactivity or methamphetamine-induced conditioned place preference. (A) No difference in velocity during habituation sessions in GFP and TeLC females (unpaired t-test, $p > 0.05$) or GFP and TeLC males (unpaired t-test, $p > 0.05$). (B) No difference has been observed on saline (S) or methamphetamine (M) sessions in GFP and TeLC females (two-way ANOVA, $p > 0.05$) or GFP and TeLC males (two-way ANOVA, $p > 0.05$). A significant effect of session is found for both males and females (two-way ANOVA, all $p < 0.0001$). (C) On testing day, neither female nor male GFP and TeLC animals show any difference on subtracted CPP score (Females - unpaired t-test, $p > 0.05$; Males - unpaired t-test, $p > 0.05$), or CPP score (Females - unpaired t-test, $p > 0.05$; Males - unpaired t-test, $p > 0.05$). (D) No difference is observed between GFP and TeLC female or male animals on time spent in METH-paired chamber pretest (Pre) and posttest (Post) (two-way ANOVA, all $p > 0.05$), but a significant effect from session has been found (two-way ANOVA, all $p < 0.0001$). Data represented as mean \pm s.e.m.

3.2.3 The role of VIPR2-expressing CeA neurons in methamphetamine-induced conditioned place preference and hyperactivity

Lastly, the role of VIPR2-expressing CeA neurons in the hyperactive and rewarding properties of methamphetamine was assessed. VIPR2-IRES-cre animals were injected in the CeA with cre-dependent TeLC or GFP (Figure 3.6). No differences were found between GFP and TeLC animals in methamphetamine-induced hyperactivity in females (Figure 3.7; two-way ANOVA, $p > 0.05$). A significant effect of session was found on velocity in both GFP and TeLC females (two-way ANOVA, $p < 0.0001$), and post-hoc tests revealed significant differences when comparing velocity on methamphetamine sessions vs corresponding saline sessions; S1 vs M1 ($p = 0.0394$), S2 vs M2 ($p = 0.0054$), S3 vs M3 ($p < 0.0001$) and S4 vs M4 ($p < 0.001$) are significantly different in GFP females (Sidak's multiple comparison test). Comparison of velocity in male GFP and TeLC animals revealed a significant effect of session as well, with post-hoc tests showing differences in S1 vs M1 ($p = 0.0299$) in TeLC males, while S4 vs M4 ($p = 0.0136$) are significantly different for GFP males (Sidak's multiple comparison tests). In addition, an interaction effect of session \times GFP/TeLC was found (two-way ANOVA, $p = 0.0029$), with significant differences shown with post-hoc tests on M2 and M4 ($p = 0.0036$, $p = 0.0243$).

These findings show that inhibition of VIPR2-expressing neurons affects methamphetamine-induced hyperactivity. No effect was found from VIPR2 inhibition on the rewarding properties of methamphetamine in either males or females in conditioned place preference (Figure 3.4D,F; unpaired t-test, $p > 0.05$), suggesting no role for VIPR2-expressing CeA neurons in the rewarding properties of methamphetamine.

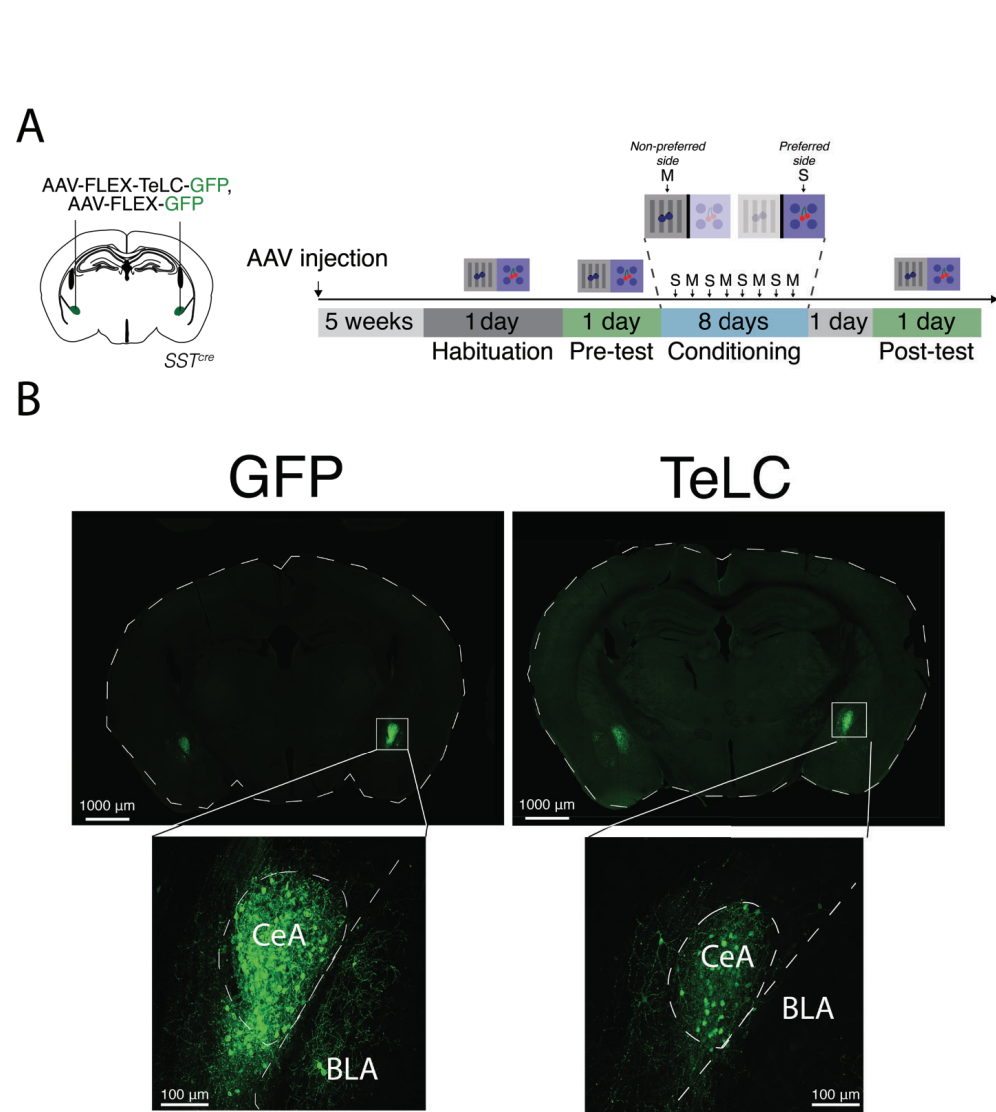


Figure 3.6. Expression of TeLC and GFP virus in VIPR2-expressing CeA neurons. (A) Schematic of experimental methods. (B) Representative histology images of a GFP (left) and TeLC (right) injected animal.

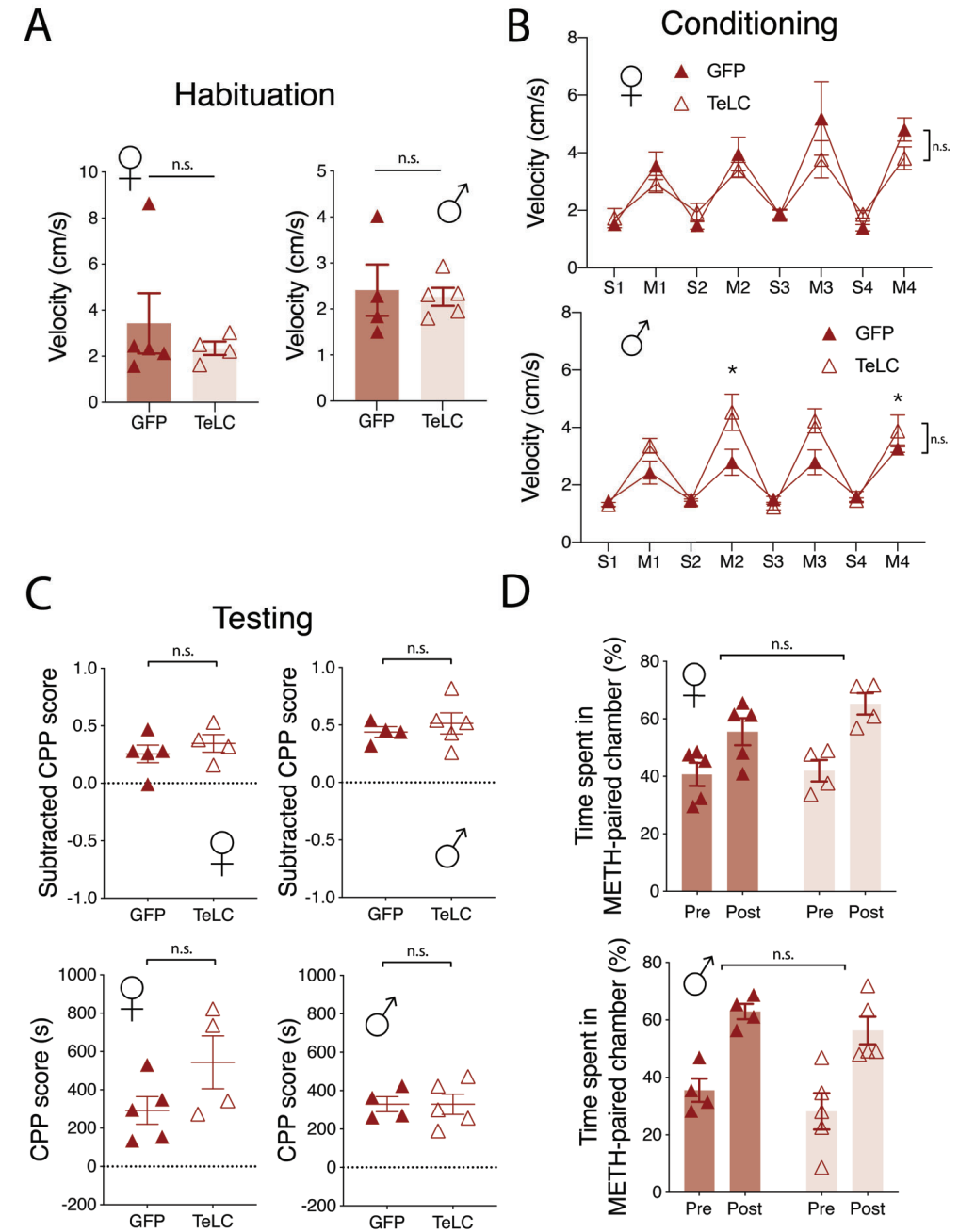


Figure 3.7 (previous page) VIPR2-expressing CeA neurons might be involved in methamphetamine-induced hyperactivity in males, but are not involved in the rewarding properties of methamphetamine. (A) No difference was found between baseline velocity during habituation between GFP and TeLC animals. (B) No difference is observed on velocity on saline (S) or methamphetamine (M) conditioning sessions between GFP and TeLC males or females (two-way ANOVA, $p>0.05$), however, a significant interaction effect of session \times GFP/TeLC was observed (two-way ANOVA, $p=0.0029$). In addition, a significant effect of session is found in both males and females (two-way ANOVA, $p<0.0001$). (C) No differences are found on subtracted CPP score and CPP score between GFP and TeLC females or males (unpaired t-tests, all $p>0.05$). (D) No difference is observed between GFP and TeLC female or male animals on time spent in METH-paired chamber pretest (Pre) and posttest (Post) (two-way ANOVA, all $p>0.05$), but a significant effect from session has been found (two-way ANOVA, males - $p<0.0001$, females - $p=0.0014$). Data represented as mean \pm s.e.m.

3.3 Methamphetamine-induced anxiety

To examine the effect of methamphetamine on anxiety, animals were exposed to the elevated plus maze (EPM). The EPM is a behavioral paradigm that is used to assess anxiety in rodents, which utilizes an animal's innate fear of open areas and the conflicting urge to explore. The EPM has closed and open arms that the animal can explore, and the time an animal will spend on the open arms will be used as a correlate of how anxious an animal is. Animals are run on the EPM for a naive baseline session first, followed by a methamphetamine session after an intraperitoneal methamphetamine injection, at least 10 days after the naive test. SST-IRES-cre, Prkcd-IRES-cre and VIPR2-IRES-cre animals are again injected in the CeA with cre-dependent TeLC or cre-dependent GFP viruses, and are behaviorally tested at least 5 weeks after injection. In our hands, methamphetamine very robustly induced anxiety-like behaviors (Figure 3.8 - 3.10).

3.3.1 The role of SST-expressing CeA neurons in methamphetamine-induced anxiety

The role of SST-expressing CeA neurons in the anxiogenic effects of methamphetamine was assessed. SST-cre animals were injected in the CeA with cre-dependent TeLC or GFP. No differences were found between GFP and TeLC female animals during either naive or methamphetamine session on time in open arms or velocity (Figure 3.8A; Two-way ANOVA, $p>0.05$). Similar results were found in males, with no difference between GFP and TeLC males during either naive or methamphetamine session on time in open arms or velocity (Figure 3.8B; Two-way ANOVA, $p>0.05$). These results demonstrate no role for SST-expressing CeA neurons in baseline anxiety levels, or methamphetamine-induced anxiety.

3.3.2 The role of Prkcd-expressing CeA neurons in methamphetamine-induced anxiety

The role of Prkcd-expressing CeA neurons in the anxiogenic effects of methamphetamine was assessed. Prkcd-IRES-cre animals were injected in the CeA with cre-dependent TeLC or GFP. No differences were found between GFP and TeLC animals during either naive or methamphetamine session on time in open arms or velocity (Figure 3.9A; two-way ANOVA, $p>0.05$). Similarly, male GFP and TeLC animals did not significantly differ on time spent in open arms (Figure 3.9B; two-way ANOVA, $p>0.05$). However, a significant difference was found between GFP and TeLC males on velocity (Figure 3.9B; two-way ANOVA, $p=0.0405$). Post-hoc comparison did not reveal any significant difference during any of the sessions, but there was a trend towards a reduction in velocity in male TeLC animals during the methamphetamine session compared to the GFP animals (Figure 3.9B; Sidak's multiple comparisons test, $p=0.0647$). This trend could be explained by two GFP animals that exhibited a relatively high velocity. However, since there is no statistical difference, it is not very likely that Prkcd neurons are involved in

velocity on an EPM after methamphetamine injection in either males or females.

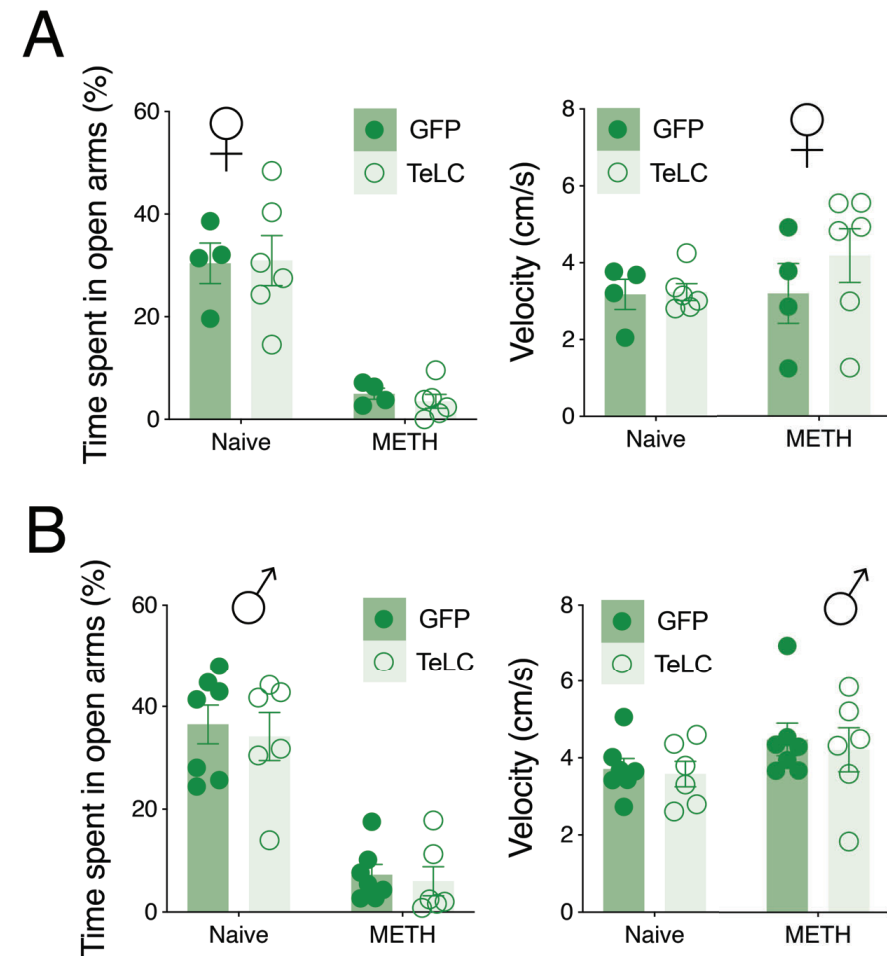


Figure 3.8 SST-expressing neurons are not involved in naive or methamphetamine-induced anxiety levels. (A) No difference is observed between GFP and TeLC females between time spent in open arms or velocity in naive state, or after methamphetamine injection (two-way ANOVA, $p>0.05$). (B) No difference is seen between GFP and TeLC male animals between time spent in open arms or velocity in naive state, or after methamphetamine injection (two-way ANOVA, $p>0.05$). Data represented as mean \pm s.e.m.

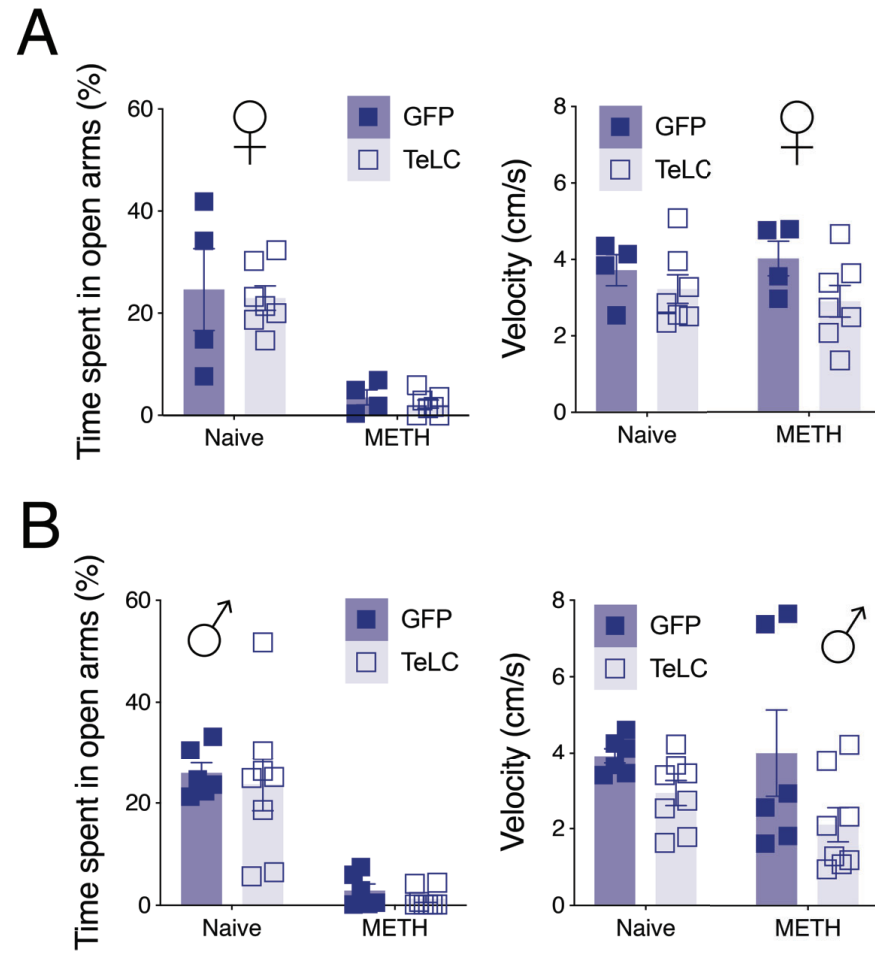


Figure 3.9 Prkcd-expressing neurons are not involved in naive or methamphetamine-induced anxiety levels. (A) No difference is observed in females between time spent in open arms or velocity in naive state, or after methamphetamine injection (two-way ANOVA, $p > 0.05$). (B) No difference is seen in males between time spent in open arms in naive state, or after methamphetamine injection (two-way ANOVA, $p > 0.05$). A significant difference is found between male GFP and TeLC animals when compared on velocity (two-way ANOVA, $p = 0.0405$). Data represented as mean \pm s.e.m.

3.3.3 The role of VIPR2-expressing CeA neurons in methamphetamine-induced anxiety

Lastly, the role of VIPR2-expressing CeA neurons in methamphetamine-induced anxiety was assessed. VIPR2-IRES-cre animals were injected in the CeA with cre-dependent TeLC or GFP. No difference was found between female (Figure 3.10A; two-way ANOVA, $p > 0.05$) or male (Figure 3.10B; two-way ANOVA, $p > 0.05$) GFP or TeLC animals on either time spent in open arms or velocity in naive session or after methamphetamine injection. These results

show that VIPR2-expressing CeA neurons are likely not involved in baseline anxiety levels, or methamphetamine-induced anxiety.

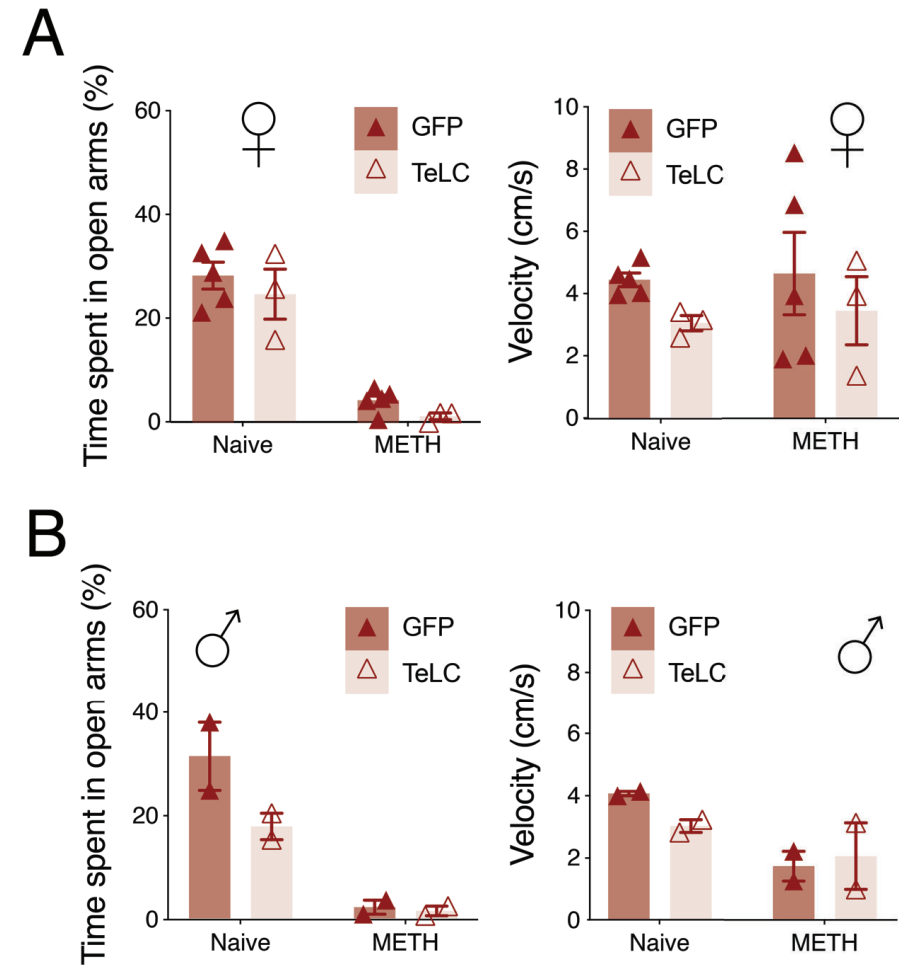


Figure 3.10 VIPR2-expressing neurons are not involved in naive or methamphetamine-induced anxiety levels. (A) GFP or TeLC females do not significantly differ between time spent in open arms or velocity in naive state, or after methamphetamine injection (two-way ANOVA, $p > 0.05$). (B) No difference is observed in male GFP and TeLC animals between time spent in open arms or velocity in naive state, or after methamphetamine injection (two-way ANOVA, $p > 0.05$). Data represented as mean \pm s.e.m.

3.4 Discussion

This study examined the role of several CeA neuronal types, namely SST-expressing, Prkcd-expressing and VIPR2-expressing neurons, in the rewarding effects of methamphetamine. Inhibition of these neuronal types did not affect the rewarding effects of methamphetamine in neither males nor females, suggesting that these cells do not mediate these behaviors in either sexes. Our results suggest that the CeA is not significantly involved in the rewarding properties of methamphetamine.

Furthermore, this study showed that methamphetamine could robustly induce anxiety-like behaviors in both male and female animals. We showed that inhibition of either of the three neuronal types in the CeA, SST-expressing, Prkcd-expressing and VIPR2-expressing neurons, does not alter the anxiogenic effect of methamphetamine, which suggests that these cells do not mediate this behavior. In addition, there was no effect from cell-type inhibition in any group on baseline anxiety levels, which shows that these cells were also not involved in baseline anxiety levels on the EPM.

Apart from the conclusion that these neurons are not involved in the acute effects of methamphetamine, it is also possible that there were some technical limitations. By using a tetanus toxin virus, we are permanently inhibiting all synaptic outputs from these neurons. It is possible that there are compensatory mechanisms at play that take over the lost function from the inhibition of these neurons. Additionally, not all neurons were infected with TeLC (by estimate 50-90% infection rate) and the presence of a minority of intact neurons may have been sufficient to mediate behavior. Moreover, a combination of the different neuronal types might be responsible for the behavior, and by inhibiting one population, the others take over. These are all possibilities, but it is likely that these neurons are not involved in the rewarding, anxiogenic or hyperactive properties of methamphetamine. These results are in concordance with one lesion study, and might explain the lack of research on this subject since then⁹⁰. Other previous studies that might have pointed towards a role for the CeA in the rewarding effects of methamphetamine were done with more gross manipulations were less precise than cell-specific inhibitions.

Even though the different cell types in the CeA do not seem to be involved in either the rewarding or anxiogenic properties of methamphetamine, several studies have demonstrated that the CeA is involved in later stages of methamphetamine dependence. Inhibition of CeA has been shown to reduce psychostimulant craving as assessed by self-administration paradigms followed by a period of abstinence (incubation of craving)¹⁰⁰⁻¹⁰². Furthermore, recently the anterior insula-input onto the CeL has been implicated in relapse¹⁰³, and the same research group has showed c-fos expression in SST-expressing CeL neurons after relapse, and c-fos expression in Prkcd-expressing CeL neurons during social-choice-induced abstinence¹⁰⁴. These studies show that the CeA, and both SST-expressing and Prkcd-expressing neurons, are involved in different stages of methamphetamine dependence. Since the VIPR2-expressing CeA neurons have some overlap with both these cells, it might also be interesting to examine further how all these different cell types together are mediating different aspects of methamphetamine dependence.