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Age- and sex-dependent effects of methamphetamine on cognitive flexibility and 5-HT_{2C} receptor localization in the orbitofrontal cortex of Sprague-Dawley rats

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Abstract

Adolescents and females experience worse outcomes of drug use compared to adults and males. This could result from age- and sex-specific consequences of drug exposure on brain function and cognitive behavior. In the current study, we examined whether a history of intravenous methamphetamine (METH) self-administration impacted cognitive flexibility and 5-HT_{2C}R localization in the orbitofrontal cortex (OFC) in an age- and sex-dependent manner. Strategy shifting was assessed in male and female Sprague-Dawley rats that had self-administered METH (0.08 mg/kg/inf) or received non-contingent infusions of saline during periadolescence or young adulthood. After all rats reached adulthood, they were tested in an operant strategy shifting task and their brains were subsequently analyzed using immunofluorescence to quantify co-localization of 5-HT_{2C} receptors with parvalbumin interneurons in the OFC. We found that adolescent-onset females were the only group impaired during discrimination and reversal learning, but they did not exhibit changes in localization of 5-HT_{2C} receptors. In contrast, adult-onset males exhibited a significant increase in co-localization of 5-HT_{2C} receptors within parvalbumin interneurons in the left hemisphere of the OFC. These studies reveal that age and sex differences in drug-induced deficits in reversal learning and 5-HT_{2C}R co-localization with parvalbumin interneurons are dissociable and can manifest independently. In addition, these data highlight the potential for certain treatment approaches to be more suitable in some populations compared to others, such as alleviating drug-induced cognitive deficits as a focus for treatment in adolescent females.

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Keywords

Adolescence; Sex differences; Cognitive flexibility; Orbitofrontal cortex; Serotonin-2C receptors; Parvalbumin

1. Introduction

Females and adolescents disproportionately account for users that experience poor outcomes of drug use (Hillhouse et al., 2007; Rawson et al., 2007; Dluzen and Liu, 2008; Chen et al., 2009) and in these populations methamphetamine (METH) use is especially problematic (Gonzales et al., 2008). Females initiate METH use at younger ages and transition to disordered use more rapidly than males (Sommers et al., 2006; Dluzen and Liu, 2008), which is of particular concern given that METH use during adolescence is associated with increases in risky sexual behaviors, teen pregnancy, and the use of other drugs (Embry et al., 2009). Moreover, the use of METH among adolescents has remained constant in recent years even though the rates of alcohol and tobacco use have declined (SAMHSA, 2014). To better understand and address the potentially unique complications of METH and other substance misuse in adolescents and females, a more complete understanding of drug-induced cognitive deficits and neuroadaptations in these populations is needed. In fact, one approach that has been used to understand differential vulnerability to substance use disorders (SUDs) has been investigation of the mechanisms underlying drug-induced cognitive dysfunction (Selby and Azrin, 1998; Rogers and Robbins, 2001; Cadet and Bisagno, 2016; Hankosky and Gulley, 2016), particularly since deficits in working memory, attention, and inhibitory control are associated with poorer treatment outcomes (Aharonovich et al., 2003, 2006; Streeter et al., 2008; Verdejo-García et al., 2012).

In laboratory rodents, psychostimulant exposure during adolescence induces similar (Hankosky et al., 2013; Hammerslag et al., 2014) or more severe (Vorhees et al., 2005; Harvey et al., 2009; Counotte et al., 2011; Sherrill et al., 2013) cognitive impairments than exposure during adulthood (for review see Hankosky and Gulley, 2016). Disrupted inhibitory control of prefrontal cortex (PFC) neural circuits has been hypothesized to underlie drug-induced cognitive deficits (Enomoto et al., 2011; Gonzalez-Burgos et al., 2011) and recent work from our lab is consistent with this. Using *in vitro* whole-cell recording we found that repeated amphetamine exposure during adolescence attenuated D₁ receptor-mediated inhibition in medial PFC pyramidal neurons in adulthood (Kang et al., 2016a; Paul et al., 2016), potentially through reductions in D₁ receptor expression (Kang et al., 2016b). The important role of adaptations in medial PFC D₁ receptors in drug-induced cognitive dysfunctions was previously described by Fletcher et al. (2007) who demonstrated that amphetamine-induced deficits in attention were alleviated by intra-PFC infusion of a D₁ receptor agonist.

In addition to dopamine, serotonergic signaling is critical for normal PFC function (Puig and Gullledge, 2011; Celada et al., 2013) and is thus another target through which vulnerability to develop SUDs may be influenced (Kirby et al., 2011). Modulation of inhibition in the PFC by serotonin is accomplished via multiple receptor subtypes that regulate excitability in

a variety of neuronal populations (Puig et al., 2005; Celada et al., 2013; Leiser et al., 2015). This inhibitory modulation is mediated in part by 5-HT_{2C} receptors (5-HT_{2C}Rs; Feng et al., 2001), likely due to their preferential expression on parvalbumin-immunoreactive (PV-ir) interneurons (Liu et al., 2007). These interneurons are the most abundant inhibitory neuron subtype in the PFC and they provide tight regulatory control of pyramidal cell output to subcortical structures (Kubota and Kawaguchi, 1994; Markram et al., 2004). As such, the preferential expression of 5-HT_{2C}Rs on PV neurons suggests that the behavioral effects associated with influencing their function may be the result of modulating inhibitory tone in the PFC. Reversal learning is one behavior that is sensitive to both 5-HT_{2C}R manipulations (Boulougouris et al., 2008; Boulougouris and Robbins, 2010) and disrupted inhibitory transmission (Bissonette et al., 2015) in the orbitofrontal cortex (OFC), and is known to be impaired following repeated exposure to amphetamine or METH (Izquierdo et al., 2010; Parsegian et al., 2011; Hankosky et al., 2013; Cox et al., 2016).

There is indirect behavioral evidence to suggest that repeated METH exposure changes 5-HT_{2C}Rs (Napier and Istre, 2008; Graves and Napier, 2012), but the impact of this exposure on 5-HT_{2C}R expression in the OFC is unknown. Based on the cellular distribution of 5-HT_{2C}Rs in the PFC (Liu et al., 2007) and evidence that antagonism of these receptors in the OFC improves reversal learning (Boulougouris and Robbins, 2010), we hypothesized that drug-induced increases in the co-localization of 5-HT_{2C}Rs on PV-ir interneurons could be a mechanism through which drug-induced deficits in cognitive flexibility manifest. To test this hypothesis, we examined strategy shifting and 5-HT_{2C}R co-localization with PV-ir interneurons in the OFC in rats with a history of intravenous METH self-administration that was initiated in adolescence or adulthood.

2. Methods

2.1 Subjects

Subjects were a total of 82 Sprague-Dawley rats (32 male, 50 female) that had previously been trained to self-administer *D*-methamphetamine HCl (0.08 mg/kg) or received non-contingent infusions of saline beginning during either adolescence or adulthood. The full methods outlining treatment of the METH rats used in this study and analysis of age- and sex-differences in METH self-administration was reported previously (Hankosky et al., 2018). Of the 46 rats that initiated METH self-administration, 15 males and 25 females met acquisition criteria to be included in this study. The remaining male (n=17) and female (n=25) rats were littermates of the METH groups treated identically with the exception that they received non-contingent infusions of saline approximated to match the number of infusions earned by their METH counterparts. Briefly, subjects were offspring of rats bred in our colony, weaned on postnatal day (P) 22, and housed 2–3 per cage. Rats were housed on a 12 h light/dark cycle (lights off at 0900) and behavior was assessed during the dark cycle (0900–1700). Rats were implanted with indwelling jugular vein catheters on P32 or 82 ($\pm$ 2 days) and had daily 2-h access to METH under increasing fixed ratio (FR) schedules of reinforcement (FR1 to FR5) or received non-contingent infusions of saline between P41–55 or P91–105, for adolescent- and adult-onset groups, respectively (Fig. 1). The last 7 days of self-administration (P56–62 and P106–112) consisted of four daily progressive ratio sessions

(5-h) each separated by one 2-h maintenance session with METH available on an FR5 schedule. Rats were allowed *ad libitum* access to food and water except during the limited periods (4 h) noted below. Experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois, Urbana–Champaign, and were consistent with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.3 Apparatus

Strategy shifting took place in standard operant chambers (Coulbourn Instruments, Whitehall, PA) that were enclosed in sound-attenuating cubicles. The cubicles were equipped with fans that provided ventilation and masked extraneous noise. One wall of each operant chamber was equipped with a centrally located food trough outfitted on either side with retractable levers that were equidistant (87 mm) from the trough. White cue lights were located above each lever and a 2.9 kHz Sonalert speaker was located directly above the food trough. A white houselight was located near the chamber ceiling on the wall opposite the nosepoke ports. Graphic State software (v3.1; Coulbourn Instruments) was used for automated chamber control and data collection.

2.4 Strategy Shifting

Five days after completion of self-administration sessions (on P67 or P117), male ($n = 6$ –10/group) and female ($n = 10$ –15/group) rats that received non-contingent infusions of saline or met acquisition criterion for METH self-administration (unit dose: 0.08 mg/kg/inf) began training for strategy shifting (Fig. 1). Food was removed 2 h prior to each daily session and returned 30 min to 2 h following its completion; rats were not otherwise food restricted and this procedure has no overall impact on body weight compared to rats fed *ad libitum* (Westbrook et al., in press). As we have described previously (Hankosky et al., 2013), rats were first trained across 4 sessions to lever press for a 45-mg food pellet (BioServ, F0021, Frenchtown, NJ). During the next 5 sessions, rats had to press the lever within 10-sec of its extension into the chamber to earn reinforcement. Subsequently, each rat's side bias was then established in a single session as described by Floresco et al. (2008).

Daily strategy shifting sessions consisted of 120 trials that were separated by a 10-sec intertrial interval. Trials began when both levers were inserted into the chamber concurrent with illumination of one of the two cue lights located above the levers. Trials ended after either the rat pressed a lever or 10 sec elapsed. Individual cue lights were presented pseudorandomly across trials, such that no cue light could be presented on more than two consecutive trials. Because all rats had prior exposure to cue lights during self-administration, the cue light illuminated 2-sec prior to trial onset during visual strategy sessions in order to increase its saliency (Floresco et al., 2008).

Rats were first trained to use a visual strategy by reinforcing them (with delivery of a food pellet) for pressing the lever that had a cue light illuminated above it, regardless of the lever's spatial location (i.e., left or right side of pellet delivery trough). Trials continued until rats achieved eight consecutive correct choices and had completed at least 30 trials. On the day following acquisition of this performance criterion, rats were required to shift to an

egocentric response strategy. This required that rats press a lever relative to its position, regardless of cue light location (e.g. always press the left lever). The location of the reinforced lever for the response strategy was determined individually for each rat such that it was the lever opposite of the rat's side bias that was observed during initial training (see above). Trials continued until rats achieved a criterion of eight consecutive correct choices.

Following acquisition of the response strategy, rats were overtrained on this response during a single session consisting of 150 trials. One day following the overtraining session, rats completed a within-session reversal of the learned response. During this session, rats were first required to demonstrate retention of the overtrained response by pressing the appropriate lever eight consecutive times. Once retention of the overtrained response was confirmed, the stimulus-reward association was reversed such that pressing on the previously non-rewarded lever now resulted in reinforcement (e.g. always press the right lever). As before, trials continued until rats achieved a criterion of eight consecutive correct choices. For all stages of the task, trials where rats failed to respond on a lever within 10-sec of trial onset were terminated, scored as an omission, and led to the intertrial interval.

2.5 5-HT_{2C}R co-expression with PV-ir interneurons

A subset of rats that completed strategy shifting ($n = 6/\text{group}$) were matched for cumulative METH intake and used for immunohistochemical analysis of 5-HT_{2C}R co-expression with PV-ir cells. Rats were chosen for this analysis based on cumulative METH intake, such that groups did not differ significantly (Fig. 2). Seven to ten days following completion of operant strategy shifting (at $P96 \pm 4$ or $P146 \pm 4$), which was ~5 weeks after the last METH self-administration session, rats were deeply anesthetized (100 mg/kg pentobarbital) and transcardially perfused with phosphate buffered saline (PBS; 10 mmol/L; pH 7.2) and 3% paraformaldehyde in PBS. Their brains were removed and post-fixed for at least 2 h at room temperature in 3% paraformaldehyde in PBS, followed by immersion in PBS containing 30% sucrose at 4 °C for 3 days until the brains sunk. Subsequently, a freezing microtome was used to collect 40- μm thick sections including the OFC and these were stored in cryoprotectant (30% sucrose, 0.1% polyvinylpyrrolidone-40, and 30% ethylene glycol in 10mmol PBS) at -20 °C until processed.

For each animal, 2 free-floating, anatomically matched coronal sections (40 μm) containing the OFC (between +3.72 and +4.68 from bregma; Fig. 5-A) were stained with 5-HT_{2C}R antibody and the calcium-binding protein antibody, PV, as described previously (Liu et al. 2007). The anti-5HT_{2C} polyclonal antibody raised in goat (1:100 dilution; sc-15081, Santa Cruz Biotechnology) and the anti-PV monoclonal antibody raised in mouse (1:2500 dilution; PV 235, SWANT) were used with fluorescent secondary antibodies Alexa Fluor® 488 (anti-goat, 1:500 dilution; A-11055 Invitrogen Corporation) and Alexa Fluor® 555 (anti-mouse, 1:2000 dilution; A-31570, Invitrogen Corporation) to visualize 5-HT_{2C}R expression and co-localization to PV-ir interneurons (Bubar et al., 2005; Liu et al., 2007). Sections were rinsed out of cryoprotectant with tris-buffered saline (TBS: pH 7.6) 5 times for 10 min each. Sections were then blocked in TBS containing 20% normal donkey serum, 1% bovine serum albumin and 1% hydrogen peroxide for 60 min at room temperature. Sections were then incubated in a primary antibody solution, diluted with TBS containing 2% normal donkey

serum and 0.3% triton-x-100, for 44 hours at 4°C. Then, sections were rinsed 6 times in TBS (6 min each) and incubated in a secondary antibody solution (diluted in TBS) for 60 min at room temperature. Following secondary antibody incubation, sections were rinsed 3 times in TBS (10 min each) and mounted on gelatin-coated slides with Vectashield reagent with a DAPI counterstain (H-1200, Vector Laboratories). To rule out secondary antibody cross-reactivity, control sections were stained with the secondary antibodies in the presence of only one primary antibody. Exclusion of an individual primary antibody resulted in no observable immunoreactivity for that protein. Images were collected using a 20× objective with a fluorescent confocal microscope (Zeiss LSM 880) and processed with the Zen software package. Sixty PV-ir cells from layer V of the lateral OFC of each rat were counted from 1–2 sections (bregma +4.2 mm). We chose to count from the lateral OFC due its role in reversal learning (Izquierdo, 2017). The number of PV-ir cells that also co-expressed the 5-HT_{2C}R was then counted and the percentage of co-localized cells was calculated.

2.6 Data Analysis

Cumulative METH intake was calculated by multiplying the unit dose of METH (0.08 mg/kg) by the number of infusions earned and summing across all days of self-administration. It was analyzed using two-way ANOVA (age × sex). Dependent measures used to assess strategy shifting included the number of trials and errors to criterion. Errors following the shift to the response strategy were classified as perseverative or never-reinforced. Perseverative errors were defined as those that would have been correct when the visual strategy was in effect (e.g., responding in the left port when the left cue light was illuminated, but the right port response was the reinforced response). All other errors were scored as never-reinforced, as they would not have been correct in a visual strategy and they included responses in the non-reinforced port during the response strategy. Because drug-induced deficits are sometimes evident early rather than later in training (Kondrad and Burk, 2004; Hankosky and Gulley, 2013; Hankosky et al., 2013), errors during the first 30 trials of the shift to response and response reversal were analyzed. Trials and errors for each strategy were analyzed in three-way ANOVAs (treatment × sex × age-of-onset). Error types were analyzed separately in males and females using three-way repeated measures ANOVA (treatment × age × type). Pre-planned comparisons were used to investigate *a priori* hypotheses that METH would impair PFC-sensitive cognition (Hankosky and Gulley, 2013; Hankosky et al., 2013).

For 5-HT_{2C}R localization, the dependent measure was percent of PV-ir interneurons that co-expressed 5-HT_{2C}Rs (number of PV-ir cells co-expressing 5-HT_{2C}Rs divided by the number of PV-ir neurons counted * 100). Sixty PV-ir interneurons were counted for each rat. Percent co-localization was analyzed with a four-way repeated measures ANOVA (treatment × sex × age-of-onset × hemisphere). Significant main effects and interactions were followed up with Tukey's post-hoc tests. The percent of 5-HT_{2C}Rs co-localized to PV-ir interneurons was correlated using simple linear regression with METH intake and errors committed early (first 30 trials) in reversal learning. For 6 rats, all PV-ir interneurons were counted from only one hemisphere.

3. Results

3.1 Strategy Shifting

Training in an operant strategy shifting task began 5 days after the last session in which rats received non-contingent infusions of saline or self-administered METH (Fig. 1). For the latter groups of rats, cumulative METH intake was not significantly different (Fig. 2). Three-way ANOVA of trials and errors to criterion during visual strategy acquisition revealed no significant main effects or interactions. However, pre-planned comparisons revealed that adolescent-onset females with a history of METH self-administration required significantly more trials ($t_{46} = 2.11$; $p = 0.0381$) and committed significantly more errors ($t_{46} = 2.02$; $p = 0.0467$) than their sex- and age-matched controls (Fig. 3-A,B).

Cognitive flexibility was then assessed by requiring rats to shift from a visual to a response strategy. Three-way ANOVA of trials to criterion (Fig. 3-C,D) revealed no significant main effects or interactions. Three-way ANOVA of errors to criterion, however, revealed a significant treatment by sex interaction ($F_{1,74} = 4.21$; $p < 0.0464$), such that control males made significantly more errors during the shift from visual to response than control females (Fig. 3-D). Errors that occurred during the shift were characterized as either perseverative or never reinforced. For males and females, three-way repeated measures ANOVAs revealed no significant main effects or interactions (data not shown). There were also no significant main effects or interactions when performance during the first 30 trials (early training) was assessed (data not shown).

Reversal learning, which is known to be sensitive to OFC function (Birrell and Brown, 2000), was next tested by overtraining and then reversing the response strategy. Three-way ANOVA of trials (Fig. 4-A) and errors (Fig. 4-B) to criterion revealed no significant main effects or interactions. However, when performance during the first 30 trials (early training) was examined, three-way ANOVA revealed a significant treatment by sex by age-of-onset interaction ($F_{1,74} = 6.36$; $p = 0.0138$). Specifically, early in training adolescent-onset females committed significantly more errors than their age-matched controls and adult-onset females (Fig. 4-C). In contrast, adult-onset females committed significantly fewer errors than their controls.

3.2 5-HT_{2C}R co-expression with PV-ir interneurons

A subset of brains from rats that completed the strategy shifting experiment were stained for 5-HT_{2C}Rs and the calcium binding protein PV (Fig. 5). Four-way repeated measures ANOVA revealed a significant treatment by sex by age-of-onset by hemisphere interaction ($F_{1,34} = 4.24$; $p = 0.0472$). In the left hemisphere, adult-onset males had significantly higher co-localization of 5-HT_{2C}Rs with PV-ir interneurons than their controls and adolescent-onset males (Fig. 5-E). In females, there were no significant group differences in 5-HT_{2C}R co-localization with PV interneurons (Fig. 5-F). In rats with a history of METH self-administration, percent co-localization was not significantly correlated with METH intake or reversal errors committed early in training overall or when stratified by hemisphere (Fig. 6).

4. Discussion

This study used rats with a history of METH self-administration or non-contingent saline exposure to investigate the impact of age and sex on METH-induced changes in cognitive flexibility and 5-HT_{2C}R localization in the OFC. As we describe elsewhere (Hankosky et al., 2018), females in our sample acquired self-administration of this METH dose (0.08 mg/kg/inf) more readily than males and this effect was driven by low levels of acquisition in adolescent-onset males. In contrast, adults of both sexes reached higher breakpoints during progressive ratio responding than adolescents. Importantly, differences in acquisition did not translate to significant differences in intake.

In the current study, we found that the effects of METH self-administration on reversal learning and 5-HT_{2C}R localization in the OFC were dissociable and depended on age and sex. Adolescent-onset females were significantly impaired during acquisition of the visual strategy and during early stages of the reversal, compared to their saline-exposed controls. However, this difference was not associated with a change in 5-HT_{2C}R localization. We instead found that adult-onset males had significantly increased co-expression of 5-HT_{2C}Rs with PV-ir interneurons in the left hemisphere of the OFC. In addition, we found no significant correlations between cumulative METH intake, early reversal errors, and co-localization measures. Our other results suggest that these relationships may be dissociable within each group, however data in the present study were collapsed across age and sex factors to achieve a large enough sample size for meaningful correlational analyses. The dissociation between cognitive impairments and changes in 5-HT_{2C}R co-expression with PV-ir interneurons is not consistent with the hypothesis that these structural adaptations contribute to drug-induced deficits in reversal learning via modulation of OFC inhibition. Alternative possibilities for the involvement of 5-HT_{2C}Rs and PV-ir interneurons in the modulation of OFC inhibition include functional changes to the receptor (Werry et al., 2008), such as alterations in ligand affinity or constitutive activity. Although the precise neurobiological mechanisms remain unknown, our results suggest that increased susceptibility to drug-induced cognitive deficits is a potential mechanism by which adolescent females may experience worse outcomes of drug use.

4.1 Strategy Shifting

Assessment of cognitive flexibility in rats with a history of METH self-administration revealed age- and sex-dependent deficits in initial discrimination learning and reversal learning early in training. Discrimination learning depends on intact corticostriatal circuits (Winocur and Eskes, 1998; Fidalgo et al., 2014) and reversal learning relies on the OFC (McAlonan and Brown, 2003; Boulougouris et al., 2007), suggesting that METH self-administration in adolescent-onset females disrupted corticostriatal and OFC-sensitive functions. The putative deficits in adolescent-onset females is consistent with age- and sex-dependent differences in the development of corticostriatal circuits, reflecting research demonstrating that development of these circuits is ongoing during adolescence (Huttenlocher and Dabholkar, 1997; Giedd et al., 1999; Casey et al., 2000; Markham et al., 2007; Rubinow and Juraska, 2009; Willing and Juraska, 2015) and this process is different in males and females (Giedd et al., 1999; Markham et al., 2007; Willing and Juraska, 2015).

That the reversal deficit was only observed early in training is consistent with other studies showing transient amphetamine-induced deficits in PFC-sensitive tasks (Kondrad and Burk, 2004; Hankosky and Gulley, 2013; Hankosky et al., 2013). Moreover, antagonism of 5-HT_{2C}Rs in the OFC reduces the number of early reversal errors committed (Alsiö et al., 2015). Coupled with our analysis of 5-HT_{2C}R co-localization, this suggests that METH exposure may result in functional, but not structural changes to 5-HT_{2C}Rs in the OFC of adolescent females. In addition, the finding that no other METH exposed groups exhibited deficits in reversal learning is consistent with a recent report (Izquierdo et al., 2016), but inconsistent with many previous studies (Izquierdo et al., 2010; Parsegian et al., 2011; Hankosky et al., 2013; Ye et al., 2014; Cox et al., 2016). The inconsistent findings might be related to these studies differences in task difficulty, total drug intake, drug-free interval prior to testing, or the method of drug exposure (i.e., volitional vs. experimenter-administered). The age-dependency of the deficits we observed is consistent with evidence demonstrating that exposure to psychostimulants during adolescence, compared to adulthood, results in greater cognitive impairments (Harvey et al., 2009; Counotte et al., 2011; Sherrill et al., 2013; Hammerslag et al., 2014). However, other studies (Hankosky et al., 2013; Hammerslag et al., 2014) have failed to observe age-dependent deficits of psychostimulant exposure in male rats. This discrepancy may be due to methodological differences such as route of drug administration (Robinson et al., 2002; Harvey et al., 2009) and withdrawal duration prior to testing (Santucci et al., 2004; Paul et al., 2016).

Adult-onset females with a history of METH self-administration committed fewer early errors than their counterparts. Interestingly, this is consistent with two studies that reported facilitation of operant strategy reversal in males exposed to amphetamine during adolescence (Hankosky et al., 2013) and in males that self-administered cocaine during adulthood (Kantak et al., 2014). Although the cause of this facilitation is not known, it is possible that adult-onset females did not learn the original strategy as well and subsequently shifted faster due to reduced proactive interference (Sherrill et al., 2013).

4.2 5-HT_{2C}R co-expression with PV-ir interneurons

In the OFC, 5-HT_{2C}R mRNA is localized to both glutamatergic and GABAergic cell types located primarily in deep layers with some expression in intermediate and superficial layers as well (Santana & Artigas, 2017). Assessment of 5-HT_{2C}R co-localization with PV-ir interneurons revealed that in the left hemisphere, adult-onset males with a history of METH self-administration had significantly higher co-localization than their age-matched controls and adolescent-onset males. The significant impact of hemisphere was not anticipated, but is consistent with a large body of literature demonstrating laterality of monoamine levels (Staiti et al., 2011), receptor distribution (Schneider et al., 1982; Drew et al., 1986; Glick et al., 1988), innervation (Neddens et al., 2004), and function (Andersen and Teicher, 1999; Neddens et al., 2004; Sullivan et al., 2009). Increased co-expression of 5-HT_{2C}Rs with PV-ir interneurons in adult-onset males with a history of METH self-administration is consistent with previous studies demonstrating psychostimulant-induced changes in 5-HT_{2C}Rs (Filip et al., 2004; Napier and Istre, 2008; Graves and Napier, 2012) and PFC GABAergic interneurons (Mohila and Onn, 2005; Morshedi and Meredith, 2007; Cass et al., 2013; Kang et al., 2016a). Although these changes were observed in the absence of cognitive deficits, it

is possible that with a more challenging task METH-induced deficits would have been detectable (Hankosky et al., 2013; Kantak et al., 2014). However, due to the lack of cognitive impairment, the functional implication of the redistribution of 5-HT_{2C}Rs is unknown.

Age- and sex-dependent differences in the structure and/or function of serotonergic (Biver et al., 1996; Zhang et al., 1999; Moll et al., 2000; Klink et al., 2002) or GABAergic systems (Cruz et al., 2003; Tseng and O'Donnell, 2007; Cholanian et al., 2014; Gonzalez-Burgos et al., 2015) at baseline or following METH self-administration may account for why 5-HT_{2C}R upregulation on PV-ir interneurons was only observed in adult-onset males. Alternatively, increased function (e.g. constitutive activity) of 5-HT_{2C}Rs localized to PV-ir interneurons could have the same outcome on OFC inhibition as structural upregulation of receptors. Indeed, there is evidence to suggest that chronic exposure to elevated levels of 5-HT, as would occur with repeated psychostimulant exposure, increases constitutive activity of 5-HT_{2C}Rs (Devlin et al., 2004). As such, distinct mechanisms of 5-HT_{2C}R-mediated OFC dysregulation, alone or in combination with changes to other systems, could constitute alternatives to receptor upregulation that disrupt reversal learning.

4.3 Conclusion

The current examination of differences in METH-induced cognitive deficits and changes in 5-HT_{2C}R localization on PV-ir interneurons revealed dissociable interactions dependent on age and sex. Adolescent-onset females were most affected by the impact of METH self-administration on strategy shifting. This suggests that increased susceptibility to the deleterious effects of METH exposure on cognition may contribute to worse outcomes in adolescent females. In fact, a recent study in adult rats demonstrated that reduced cognitive flexibility predicted subsequent intake and escalation of METH self-administration (Istin et al., 2016). Future studies will be needed to determine the impact of drug-induced cognitive dysfunction on subsequent drug-seeking. We hypothesized *a priori* that METH-induced cognitive impairments would be associated with an upregulation of 5-HT_{2C}Rs on PV-ir interneurons, but the only group that exhibited these drug-induced neuroadaptations was adult-onset males. In the absence of cognitive deficits, the impact of these changes is unknown and future work should investigate alternative 5-HT_{2C}R adaptations that may alter inhibitory control in the OFC. One implication of our findings is that certain treatment approaches may be more beneficial in some populations of drug users than in others and that alleviating cognitive deficits may be especially efficacious in adolescent females.

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Highlights

- Drug-induced neuroadaptations and cognitive impairment depend on age and sex
- Methamphetamine self-administration impairs reversal learning in adolescent females
- Methamphetamine self-administration changes 5-HT_{2C}R localization in adult males
- Reversal learning deficits and 5-HT_{2C}R expression changes manifest independently

Figure 1.

Experimental timeline (A) and table indicating sample sizes for rats tested in the strategy shifting task (B). The timeline shows the approximate age (in days) of adolescent- and adult-onset rats for the previously reported (Hankosky et al., 2018) self-administration phase, the strategy shifting task phase, and the age when tissue was collected for immunofluorescence. Surgery and behavioral testing were done ± 2 days and tissue collection varied by ± 4 days. The table shows the subset of rats from the original sample (Hankosky et al., 2018) that met acquisition criterion for METH self-administration and were subsequently trained and tested for strategy shifting.

Figure 2. Cumulative METH intake across all sessions for individual rats used in tests of strategy shifting. All rats acquired self-administration at a unit dose of 0.08 mg/kg/inf and mean intake for each group is indicated by the horizontal line with error bars representing SEM. Individual rats indicated with a square symbol ($n = 6/\text{group}$) were those used for immunohistochemical analysis of 5-HT_{2C}R localization.

Figure 3.

Acquisition of visual strategy and shift to response strategy in males ($n=6-10/\text{group}$) and females ($n=10-15/\text{group}$) with a history of METH self-administration ($n=6-15/\text{group}$) or exposure to saline ($n=7-13/\text{group}$). Shown are trials (A) and errors (B) to reach acquisition criterion of the visual strategy and trials (C) and errors (D) to criterion during the shift from visual to response strategy. * $p < 0.05$ for indicated comparison; matching letters indicate $p < 0.05$ for the indicated sex difference collapsed across age.

Figure 4. Reversal of the overtrained response in males ($n=6-10/\text{group}$) and females ($n=10-15/\text{group}$) with a history of METH self-administration ($n=6-15/\text{group}$) or exposure to saline ($n=7-13/\text{group}$). Shown are trials (A) and errors (B) to criterion for reversal of the response strategy, as well as errors committed during the first 30 trials of the reversal (“early errors”; C). * $p < 0.05$ vs. age-matched control; ** $p < 0.01$ adolescent- vs. adult-onset.

Figure 5. 5-HT_{2C}R co-localization with parvalbumin (PV) interneurons. (A) Schematic drawing of a representative section, adapted from Paxinos and Watson (2007), from which PV-ir cells from layer V of the lateral OFC were sampled (gray boxes). Brains from a subset of rats (see Fig. 2) were stained for (B) 5-HT_{2C}R (green) and (C) PV (red). Subsequently, co-localization of the proteins was assessed (D). Arrows indicate cells co-expressing 5-HT_{2C}R and the PV calcium-binding protein (inset: enlarged image of double-labeled cell). Mean percentage of 5-HT_{2C}R co-localization with PV-ir interneurons in both hemispheres of the OFC is shown for (E) males (n = 3–6/hemisphere) and (F) females (n = 5–6/hemisphere). * $p < 0.05$.

Figure 6.

Scatterplots showing the relationship of (A,B) cumulative METH intake and (C,D) early reversal errors with percent co-localization (5-HT_{2C}Rs in PV interneurons) in the left (A,C) and right (B,D) hemisphere of the OFC in rats with a history of METH self-administration. None of these relationships were statistically significant.