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ORIGINAL ARTICLE

Deficits in behavioral sensitization and dopaminergic responses to methamphetamine in adenylyl cyclase 1/8-deficient mice

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Abstract

The cAMP/protein kinase A pathway regulates methamphetamine (METH)-induced neuroplasticity underlying behavioral sensitization. We hypothesize that adenylyl cyclases (AC) 1/8 mediate these neuroplastic events and associated striatal dopamine regulation. Locomotor responses to METH (1 and 5 mg/kg) and striatal dopamine function were evaluated in mice lacking AC 1/8 (DKO) and wild-type (WT) mice. Only 5 mg/kg METH induced an acute locomotor response in DKO mice, which was significantly attenuated versus WT controls. DKO mice showed a marked attenuation in the development and expression of METH-induced behavioral sensitization across doses relative to WT controls. While basal and acute METH (5 mg/kg)-evoked accumbal dialysate dopamine levels were similar between genotypes, saline-treated DKO mice showed elevated tissue content of dopamine and homovanillic acid in the dorsal striatum (DS), reflecting dysregulated dopamine homeostasis and/or metabolism. Significant reductions in DS dopamine levels were observed in METH-sensitized DKO mice compared to saline-treated controls, an effect not observed in WT mice. Notably, saline-treated DKO mice had significantly increased phosphorylated Dopamine- and cAMP-regulated phosphoprotein levels, which were not further augmented following METH sensitization, as observed in WT mice. These data indicate that AC 1/8 are critical to mechanisms subserving drug-induced behavioral sensitization and mediate nigrostriatal pathway METH sensitivity.

Keywords: adenylyl cyclase, DARPP-32, dopamine, *in vivo* microdialysis, locomotor sensitization, methamphetamine.
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Methamphetamine (METH) remains one of the most widely abused illicit substances in the world; surpassed only by cannabis in terms of frequency of worldwide illicit drug use (United Nations Office on Drugs and Crime 2014). The impact of METH as a major public health concern is highlighted by its high relapse potential, ability to induce pronounced dysfunctions in cognitive, behavioral, and emotional regulation with long-term use (Meredith *et al.* 2005), and the lack of current effective therapeutic options for METH addiction. METH possesses intense psychomotor activating and motivational properties associated with the activation of central dopamine systems (Itzhak *et al.* 2002; Jones *et al.* 2007; Fornai *et al.* 2009; Zombeck *et al.* 2009), where it acts as a substrate for the dopamine transporter

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Abbreviations used: 3-MT, 3-methoxytyramine; AC, adenylyl cyclase; aCSF, artificial cerebral spinal fluid; cdk5, cyclin-dependent kinase 5; COMT, catechol-*O*-methyl-transferase; DARPP-32, dopamine- and cAMP-regulated phosphoprotein; DAT, dopamine transporter; DKO, adenylyl cyclase 1 and 8 double knockout mice; DOPAC, 3,4-dihydroxyphenylacetic acid; ERK, extracellular signal-regulated kinase; HVA, homovanillic acid; i.p., intraperitoneal; LTP, long-term potentiation; MAO, monoamine oxidase; METH, methamphetamine; MSN, medium spiny neuron; NAc, nucleus accumbens; PKA, protein kinase A; VMAT, vesicular monoamine transporter; WT, wild-type mice.

(DAT) and the vesicular monoamine transporter, and is also reported to inhibit monoamine oxidase (MAO) (Sulzer *et al.* 2005; Fleckenstein *et al.* 2007; Chen *et al.* 2009). Through these mechanisms, METH profoundly stimulates dopamine elevations within striatal pathways, which acutely promotes enhanced locomotor activity (Fornai *et al.* 2009; Zombeck *et al.* 2009). With repeated exposure, METH produces behavioral sensitization manifested as a progressive enhancement of behavioral responses that persists even after long periods of withdrawal (Robinson and Berridge 1993; Vanderschuren and Kalivas 2000). The sensitization response to a drug is proposed to evoke fundamental neuroadaptive processes generalizable to aspects of human addiction, such as compulsive drug seeking behavior (Vanderschuren and Kalivas 2000; Robinson and Berridge 2008), and the circuitry, neurotransmitter, and receptor systems are overlapping between sensitization and self-administration reinstatement behaviors (Steketee and Kalivas 2011). Among the numerous neural mechanisms implicated, the cAMP/protein kinase A (PKA) pathway plays a prominent role in conferring METH-induced synaptic modifications in striatal reward neurocircuits (Moriguchi *et al.* 2002; Miyazaki *et al.* 2013). However, the candidate proteins that initiate these signals are largely undefined.

One of the best characterized substrates of dopamine and PKA signaling in the striatum is the Dopamine- and cAMP-regulated phosphoprotein (DARPP-32). DARPP-32 is enriched in medium spiny neurons (MSNs), where it integrates striatal neuronal output and serves as an important mediator of drug-induced neuroplastic changes (Svenningsson *et al.* 2004). The activation state of DARPP-32 is bi-directionally modulated by PKA and cyclin-dependent kinase 5 (cdk5), the balance of which acts as a switch in several neurotransmitter-induced signals (Svenningsson *et al.* 2004). Under basal conditions, DARPP-32 is primarily phosphorylated at Thr-75 by cdk5, which inhibits the phosphorylation of Thr-34 by PKA (Bibb *et al.* 1999; Svenningsson *et al.* 2004). Enhanced dopaminergic transmission via dopamine D1 receptors leads to a decrease in the phosphorylation of Thr-75, alleviating the inhibition of PKA-mediated DARPP-32 phosphorylation at Thr-34 that potently inhibits protein phosphatase 1 (Greengard 2001). Chronic exposure to dopamine-activating central stimulants regulates the phosphorylation state of DARPP-32 that, in turn, is an essential mediator of sensitization driven behavioral and cellular plasticity (Lin *et al.* 2002; Chen and Chen 2005; Valjent *et al.* 2005; Zachariou *et al.* 2006; Scheggi *et al.* 2007).

Adenylyl cyclase (AC) enzymes catalyze cAMP formation. Of the nine neuronal isoforms of AC enzymes, only AC 1 and AC 8 are primarily stimulated by calcium and calmodulin. AC 1 and 8 are important for several forms of experience-dependent neuroplasticity associated with learning and memory (Zhang *et al.* 2008, 2011), likely mediated by their ability to couple activity-dependent calcium increases to activation of cAMP signaling pathways (Xia and Storm 1997; Wang and Storm 2003). Both AC 1 and 8 are expressed across the striatum (Yao *et al.* 2004; Olsen *et al.* 2008; Conti *et al.* 2009b), where they were shown to functionally contribute to calcium-stimulated AC activity in rodent and human tissue (Gnegy *et al.* 1980; Ahlijanian and Cooper 1988; Tong *et al.* 2001; DiRocco *et al.* 2009). Previous evidence demonstrates a role for AC 1 and 8 in the acute neuronal and behavioral responses to abused substances, including alcohol, toluene, morphine, and cocaine (Maas *et al.* 2005; Zachariou *et al.* 2008; Conti *et al.* 2009a, 2012; DiRocco *et al.* 2009). Likewise, calcium-stimulated ACs were shown to contribute to prolonged drug effects, as evidenced by the lack of a locomotor sensitization response to chronic cocaine in AC 1/8-deficient mice (DiRocco *et al.* 2009). This behavioral effect was accompanied by impaired activation of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase signaling pathway and downstream cAMP response element-binding protein-dependent transcription, indicating the involvement of these AC isoforms in cocaine-induced striatal post-synaptic plasticity (Thomas *et al.* 2008; DiRocco *et al.* 2009). However, AC 1 and 8 are also known to be located and influence pre-synaptic regulatory mechanisms (Conti *et al.* 2007, 2009a). As striatal dopaminergic transmission is critical to mediate behavioral sensitization to stimulants (Robinson and Berridge 1993; Vanderschuren and Kalivas 2000; Thomas *et al.* 2008), AC 1/8 isoforms could modulate sensitization behavior by regulating dopamine release through calcium signaling mechanisms, a presently unexplored possibility.

This study will test the hypothesis that calcium-stimulated ACs functionally contribute to METH-induced behavioral sensitization through regulation of associated pre-and post-synaptic neuroplastic events involving dopamine in the striatum. As the presence of either AC 1 or AC 8 in single genetic knockouts compensates for loss of the other enzyme in measures of neuronal calcium-stimulated AC activity (Wong *et al.* 1999; Maas *et al.* 2005; DiRocco *et al.* 2009), this study utilized mice with a genetic deletion of both AC 1 and AC 8 (DKO). In this study, several parameters of dopamine pre-synaptic function following acute and sensitized METH administration were evaluated in WT and DKO mice using *in vivo* microdialysis (nucleus accumbens, NAc) and tissue content analysis (dorsal and ventral striatum) of dopamine and its metabolites. This study further assessed if alterations in dorsal striatal dopamine levels and locomotor sensitization to METH in DKO mice were associated with aberrant regulation of post-synaptic DARPP-32 signaling by measuring the relative levels of activated (phosphorylation of residue Thr-34) and quiescent (phosphorylation of Thr-75) DARPP-32 in this region. Together, these results reveal a previously undefined role for calcium-stimulated ACs in pre- and post-synaptic dorsal striatal dopaminergic regulation

that likely contributes to the altered behavioral responsiveness to METH.

Materials and methods

Animals

The production of DKO mice has been described previously (Wu *et al.* 1995; Wong *et al.* 1999; Wong and Storm 2002). DKO mice were backcrossed more than 10 generations onto a C57BL/6 background (C57BL/6 mice obtained from The Jackson Laboratory), with homogeneity on the C57BL/6 background established by comparison of polymorphic markers between C57BL/6 and 129 mouse strains. Progeny of DKO homozygous mutants and WT (C57BL/6) mice bred and raised in our colony were used for the present studies. Experimental procedures were performed on male mice between 8 and 12 weeks of age with age-matched WT mice used as controls. Mice were housed in standard microisolator cages maintained under controlled conditions of temperature (~24°C), light cycle (12 h light/dark cycle; lights on from 0700 to 1900 h), and humidity (35–40%). Mice were given free access to food and water throughout the duration of the study. All procedures were approved by the Wayne State University Institutional Animal Care and Use Committee. Animal care and use was in accordance with National Institutes of Health Animal Care guidelines and was overseen by the Wayne State University Division of Laboratory Animals, which maintains AAALAC accredited facilities.

METH-induced locomotor sensitization

Locomotor activity was monitored in WT and DKO mice using the Opto-M3 Activity Meter (Columbia Instruments). This computerized system consisted of an array of 16 parallel infrared (IR) emitter detector pairs mounted in novel static activity chambers, into which a standard mouse microisolator cage was placed for activity measurements. Beam breaks in the horizontal plane were recorded in 1 min intervals and used to quantify locomotor ambulatory activity and stereotypic non-ambulatory movements. Ambulatory activity was scored when a series of new IR beams were interrupted in sequence. Subtraction of ambulatory counts from the total number of IR beam interruptions was used to calculate the number of rapid, repeated beam breaks produced by stereotypic-like movements, such as head-bobbing and grooming.

On each day of locomotor recording, mice were acclimated to the experimental room for 1 h, given an injection of saline or METH, and placed in the recording chamber for 20 min. Racemic (±) methamphetamine hydrochloride (METH; NIDA Drug Supply Program, Bethesda, MD, USA) was used in all the current studies and dissolved in normal sterile saline (0.9% NaCl solution) prior to injection. On days 1–4, baseline locomotor activity was assessed in WT and DKO mice given a single daily intraperitoneal (i.p.) injection of sterile saline. Mice were then divided into groups to assess acute and sensitized locomotor responses to METH. Acute locomotor activity was measured in a group of WT and DKO mice given a single injection (i.p.) of sterile saline, 1 or 5 mg/kg METH on day 5. A separate cohort of WT and DKO mice received a single daily injection of sterile saline, 1, or 5 mg/kg METH (i.p.) on days 5–9 and were monitored for development of locomotor sensitization. After 3 drug-free days, sensitized mice were examined for expression of sensitization with a challenge dose of saline, 1, or 5 mg/kg METH (i.p.) on day 13, according to their initial treatment group. Ambulatory activity was reported as percent change relative to the saline-treated control group with respect to genotype.

Microdialysis

Microdialysis surgeries were performed as described previously (Bosse *et al.* 2012). Briefly, WT and DKO mice were anesthetized with isoflurane (3% induction, 2% maintenance) and placed on a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). Once mice were appropriately anesthetized, a CMA/7 guide cannula (Harvard Apparatus, Holliston, MA, USA) was inserted to allow dialysis sampling from the NAc (ML +0.70, AP +2.40, DV −3.60), using coordinates determined by the mouse atlas (Paxinos and Franklin 2001) and empirical refinement. Ophthalmic ointment was applied immediately after anesthetic induction to protect eyes during the surgical procedure. The animal's body temperature was maintained using a heating pad (~ 37°C). A lidocaine (0.5 mg/kg) and bupivacaine (1.5 mg/kg) mixture was subcutaneously injected around the incision site to minimize pain. The guide cannula was secured using dental cement. Following recovery (~ 3–4 h), a CMA/7 microdialysis probe (1 mm length, 0.24 mm diameter, Cuprophane, 6 kDa cut-off, Harvard Apparatus) was inserted and perfused (0.8 μL/min) with sterile artificial cerebrospinal fluid (aCSF; composition: 145 mM NaCl, 3.5 mM KCl, 2.0 mM Na_2HPO_4, 1.0 mM $CaCl_2$, 1.2 mM $MgCl_2$, and pH = 7) continuously until experimental dialysis collection the following day.

Following overnight perfusion, three baseline dialysate samples (20 min each) were collected by perfusing aCSF at 0.8 μL/min for 1 h. Mice then received a single injection of METH (5 mg/kg, i.p.) and 20 min dialysis fractions were collected with aCSF for 4 h post injection. All dialysate samples were collected into 5 μL of a stabilizing solution, containing 0.2 M perchloric acid, 0.2 μM ascorbic acid, and 0.2 μM EDTA. After dialysis, brains were removed and sectioned for histological verification of probe placement as previously described (Bosse *et al.* 2012). Dialysis dopamine levels for each mouse were normalized to their uncorrected baseline values and the average percent change was reported for each group.

Striatal tissue preparation

Tissues were prepared from WT and DKO mice following completion of the locomotor assessments (described above) for electrochemical analysis of monoamine content and immunoblot determination of DARPP-32 phosphorylation levels. WT and DKO mice were rapidly decapitated either 30 min after a single injection of METH (5 mg/kg, i.p.) in the acutely treated group or 24 h after the last METH injection on day 13 in the sensitized group. Brains were immediately removed and stored at −80°C until sectioned using a biopsy punch as described in Perrine *et al.* (2011). Briefly, brains were thawed, sectioned into 2 mm coronal slices on an ice-cold brain slice matrix, and a 1.5 mm biopsy punch was used to isolate the dorsal (primarily caudate putamen) and ventral (primarily NAc core and shell) striatum. Dissections were guided by neuroanatomical landmarks using the mouse atlas (Paxinos and Franklin 2001). For tissue content analysis, punches from the right hemisphere were homogenized in 0.1 M perchloric acid, centrifuged (7200 *g*) for 10 min, and the supernatant was immediately extracted for HPLC separation and electrochemical detection of monoamines (described in the following section). The left hemisphere punches

were homogenized in whole cell lysis buffer [composition in mM: 20 Tris (pH 7.5), 150 NaCl, 2 EDTA, 1% (v/v) Triton-X, 10% (v/v) glycerol, and protease inhibitor] for immunoblot analysis. Following homogenization, immunoblot preparations were centrifuged (10 000 *g*) for 10 min (4°C) and the supernatant stored at −80°C until analyzed.

HPLC-electrochemical analysis of monoamines

In this study, monoamine levels were assessed from both dialysate and tissue homogenates. Dialysis samples and the supernatant from sensitized mouse tissue were manually injected (20 μL) on to a Phenomenex Luna C18(2)-HST column (100 × 3.00 mm, 2.5 μm particle size) for separation followed by detection using a Coulochem III detector coupled to a 5011A high sensitivity analytical cell (E1 = −150 mV; E2 = +230 mV vs. Pd). A guard cell (ESA 5020) was used in-line before the injection loop and was set at a potential of +350 mV. Separation was achieved by a Shimadzu isocratic LC-20AD HPLC pump that delivered mobile phase (composition in mM: 75.2 sodium phosphate, 1.4 octanesulfonic acid, 0.125 EDTA, 0.002% triethylamine, and 10% acetonitrile; pH 3.0) at a flow rate of 0.38 mL/min. The supernatant from the acutely treated group was injected (10 μL) using a cooled autosampler (4°C) onto a Thermo Scientific C18-RP column (150 × 3.00 mm) for separation followed by detection using a Coulochem III detector coupled to a 5011A high sensitivity analytical cell (E1 = −175 mV; E2 = +300 mV vs. Pd). A guard cell (ESA 5020) was used in-line before the injection loop and was set at a potential of +350 mV. Separation was achieved by a Dionex pump (Thermo Scientific) that delivered TEST mobile phase (Thermo Scientific, Waltham, MA, USA) at a flow rate of 0.6 ml/min. Peak area for each analyte was integrated and quantified against known standards using Shimadzu LC solutions software, Nakagyo-ku, Kyoto, Japan; (dialysis and sensitized samples) or Dionex Chromeleon 7 software (acute samples). Absolute concentrations for each analyte were corrected for total soluable protein (BCA™ protein kit, Thermo Scientific) and normalized to percent change from the WT saline-treated control.

Immunoblot analysis

Equal amounts of whole cell protein lysates (10–20 μg) were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (4–12% Bis-Tris NuPAGE gel) and transferred to a nitrocellulose membrane. Membranes were probed with primary antibodies against pThr-34-DARPP-32, pThr-75-DARPP-32, DARPP-32 (1 : 2000, Cell Signaling Technology, Beverly, MA, USA), and actin (1 : 5000, Sigma, St Louis, MO, USA). Primary antibodies were detected using horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies and signals visualized using chemiluminescence. Densitometric analysis was performed using Image J Software (NIH, Bethesda, Maryland, USA). Phosphoprotein signals were normalized to the corresponding total DARPP-32 bands and the total DARPP-32 signals were normalized to the corresponding actin bands. The normalized signal intensity for each was then averaged within groups.

Data analysis

Data analysis was performed using Excel, SPSS (IBM, Armonk, NY, USA) and GraphPad Prism (GraphPad, La Jolla, CA, USA) software. All values were reported as mean ± SEM and the criterion for statistical significance was $p \leq 0.05$. Evaluation of genotype and time effects in the locomotor response to saline and METH-induced stereotypic movements were assessed with two-way repeated measures ANOVA in GraphPad. The acute and sensitized locomotor response to METH was evaluated using three-way repeated measures ANOVA in SPSS with between-subjects factors of genotype and treatment and within-subjects factor of time (day). As the Mauchly's test indicated sphericity was violated, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.494$). *Post hoc* analysis for group differences was performed with Fisher's least significant difference test. Two-way repeated ANOVA analysis in SPSS was used to assess dialysis dopamine changes with genotype as the between-subjects factor and time (min) as within-subjects factor with a Greenhouse-Geisser correction. Missing values in the dialysis dopamine data were handled using SPSS missing value analysis and imputation, which utilizes the expectation maximization model method. Separate group-wise two-way ANOVA comparisons in GraphPad were performed for tissue content levels of each analyte in either the acute or sensitized cohorts with genotype and treatment as the independent variables. Bonferroni multiple comparison analysis was used for post-test. Separate two-way ANOVA tests in GraphPad were used to evaluate differences in each DARPP-32 comparison with genotype and treatment as between-subject factors and Bonferroni multiple comparison as the post-test.

Results

Mice lacking AC 1/8 show a blunted acute and sensitized locomotor response to METH

Locomotor activity was measured in two groups of WT and DKO mice that were either administered saline throughout the study (days 1–9, day 13) or saline (day 1–4) followed by acute (day 5) and sensitized (day 6–9, day 13) exposure to METH. On test day 1, the average ambulatory activity recorded in DKO mice following a saline injection (2806 ± 156 ambulatory count) was significantly greater than observed in WT mice (2173 ± 165 ambulatory count) ($p < 0.01$) (data not shown). Consistent with previous findings (Wieczorek *et al.* 2010; Conti *et al.* 2012), repeated measures ANOVA confirmed that DKO mice showed a heightened locomotor response to saline compared to WT mice across all test days, reflected by a significant effect of genotype [$F(1, 31) = 16.96$, $p = 0.0003$] and time [$F(9, 279) = 47.64$, $p < 0.0001$], but no interaction [$F(9, 279) = 0.487$, $p = 0.833$] (data not shown). As a result, METH-induced locomotor activity levels were normalized to the response of saline-treated mice within each genotypic condition.

Following saline treatment on acclimation days 1–4, a progressive enhancement in the ambulatory response was observed with repeated administration of 1 mg/kg (Fig. 1a) and 5 mg/kg (Fig. 1b) METH. The acute and sensitized locomotor response to 1 mg/kg (Fig. 1c) and 5 mg/kg (Fig. 1d) METH were assessed on day 5 and day 13 (the

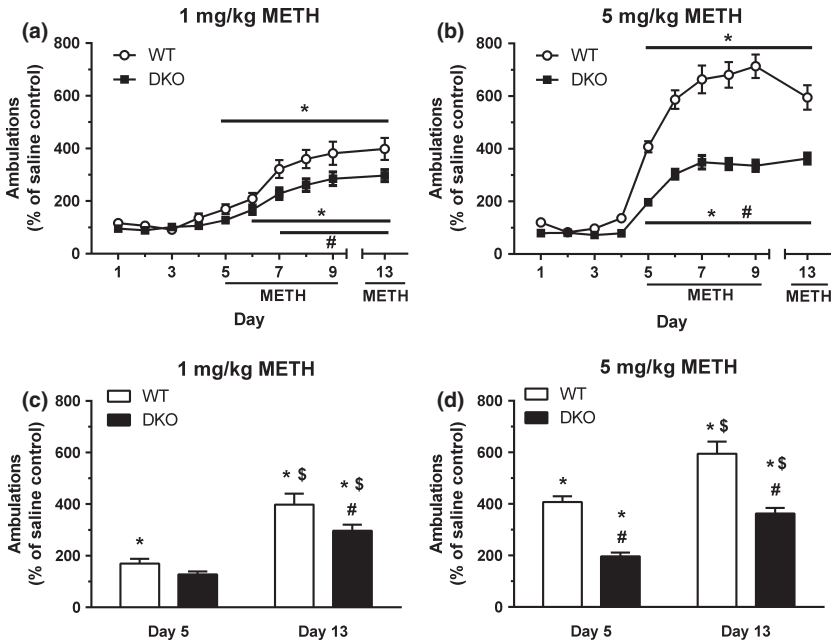

Fig. 1 Acute and sensitized locomotor response to 1 mg/kg (a and c) and 5 mg/kg (b and d) methamphetamine (METH) in wild-type mice (WT) and DKO mice (n = 16–19/group). Following acclimation to saline injections on days 1–4, locomotor activity was recorded immediately after daily saline or METH administration on day 5–9. After 3 drug-free days, a challenge injection of saline or METH was given on day 13, according to the respective treatment group. (a and b) Repeated METH exposure produced a significant increase in locomotor activity relative to the average locomotor response in saline-treated controls with respect to genotype, though this effect was significantly blunted in DKO mice compared to WT mice. (c and d) Acute METH exposure (day 5), at both doses, stimulated locomotor activity in WT mice. DKO mice only displayed an increased acute response to 5 mg/kg METH, which was attenuated compared to METH-treated WT mice. When challenged with METH (day 13), both WT and DKO mice demonstrated enhanced activity, however, this response was blunted in DKO mice. Significance was determined by three-way repeated measures ANOVA. $^{*}p < 0.001$, compared to respective saline controls (groups not shown); $^{\#}p < 0.001$, compared to METH-treated WT mice; $^{\$}p < 0.001$, compared to respective acute response (day 5).

latter was after 3 drug-free days), respectively. Repeated measures ANOVA revealed a significant genotype × treatment interaction across METH test days [$F(2.472, 224.988) = 3.492$, $p = 0.005$]. In WT mice, acute administration of METH (i.p., day 5) dose-dependently increased ambulatory activity relative to WT saline controls at both the 1 mg/kg (Fig. 1a and c) and 5 mg/kg (Fig. 1b and d) dose. In DKO mice, only the highest dose of METH tested (5 mg/kg) stimulated an enhanced locomotor response compared to DKO saline controls ($p < 0.001$), though this response was significantly blunted compared to METH-treated WT mice ($p < 0.001$) (Fig. 1b and d). Thus, DKO mice displayed an attenuated sensitivity to the locomotor stimulatory effects of acute METH compared to WT mice. Repeated administration of either 1 or 5 mg/kg METH produced a sensitized locomotor response in WT mice, as indicated by enhanced activity during the METH challenge on day 13 compared to the acute response to METH on day 5 ($p < 0.001$) (Fig. 1c and d). DKO mice also demonstrated a progressive increase in locomotor activity in response to the METH sensitization procedure at both doses ($p < 0.001$) (Fig. 1c and d). However, the locomotor response to a challenge injection of either 1 or 5 mg/kg METH following sensitization was significantly attenuated in DKO mice relative to METH-sensitized WT mice on day 13 ($p < 0.001$). Stereotypic non-ambulatory movements also increased following the 5 mg/kg METH challenge (day 13) relative to the response recorded after acute METH administration (day 5), reflected by a significant effect of time [$F(1, 64) = 52$, $p < 0.0001$] (data not shown). The expression of stereotypy behavior following repeated METH administration was similar across WT and DKO mice, as no between-subjects effect of genotype [$F(1, 64) = 0.056$, $p = 0.812$) or genotype × time interaction [$F(1, 64) = 0.026$, $p = 0.871$) was observed.

Acute METH elicits comparable stimulation of accumbal dopamine efflux in WT and DKO mice

To investigate the mechanism through which AC 1/8 regulate METH-mediated locomotor activity, METH-induced stimulation of dialysis dopamine levels in the NAc was measured in WT and DKO mice. Baseline levels (nM ± SEM) of extracellular dopamine in the NAc were similar in WT mice

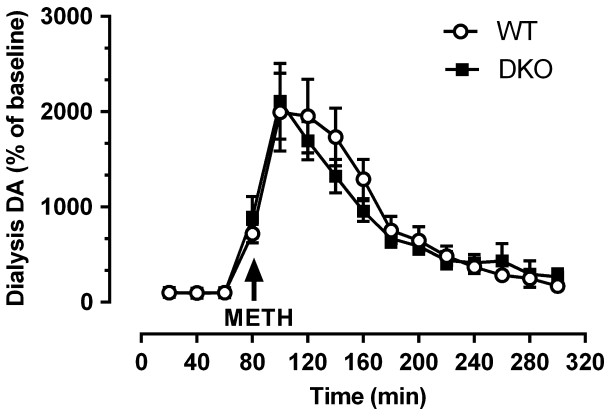

Fig. 2 Extracellular dopamine (DA) levels measured in the NAc by *in vivo* microdialysis in wild-type mice (WT) and DKO mice (n = 6–8/group). Following collection of three baseline fractions (20 min each), mice were injected with 5 mg/kg (i.p.) methamphetamine (METH) immediately prior to collection of the forth dialysis sample. METH exposure evoked a measurable increase in dialysate DA levels that was comparable across genotypes and returned to baseline levels ~ 3.5 h after injection.

(3.2 ± 0.6) and DKO mice (2.8 ± 0.4). Acute administration of 5 mg/kg (i.p.) METH significantly elevated NAc dopamine levels in both WT and DKO mice, as indicated by a significant within-subjects effect of time with repeated measure two-way ANOVA analysis [$F(1.36, 18.967) = 18.49$, $p < 0.001$] (Fig. 2). METH-induced dopamine release peaked in the second 20-min fraction following acute injection (100 min), which resulted in an approximate 200-fold increase in baseline dopamine levels in both WT and DKO mice. No between-subjects effect of genotype ($p = 0.560$) or interaction of genotype × time ($p = 0.598$) was observed, indicating the magnitude of METH-stimulated dopamine efflux in the NAc was comparable between WT and DKO mice.

METH and AC 1/8 deletion differentially effect pre-synaptic parameters of dopamine regulation

To gain a comprehensive understanding of the neuromodulatory effects of AC 1/8 on other indices of striatal dopaminergic pre-synaptic function, tissue content levels of dopamine and its metabolites were measured in saline and METH-treated WT and DKO mice. Tissue content analysis was performed in both the anterior dorsal striatum and the anterior ventral striatum (including the NAc) either 30 min following acute administration of 5 mg/kg METH (i.p., day 5) (Fig. 3a and b, respectively) or 24 h after a challenge

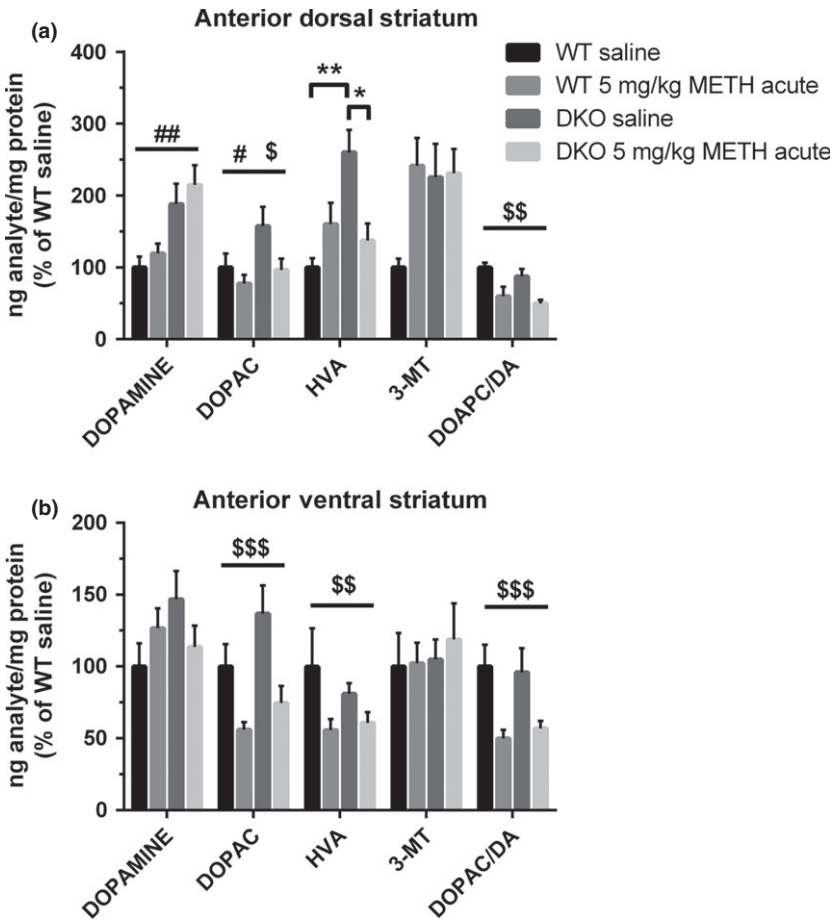

Fig. 3 Acute tissue content levels of dopamine and its metabolites measured in the (a) anterior dorsal striatum and (b) anterior ventral striatum (NAc) of wild-type mice (WT) and DKO mice (n = 6–9/group). Tissues were collected 30 min following an acute saline or methamphetamine (METH, 5 mg/kg) injection. Data reported as average percentage change over WT saline-treated controls and independent two-way ANOVA analysis was conducted for each analyte. $^{\$}p > 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$, main effect of METH treatment; $^{\#}p = 0.054$, $^{\#\#}p < 0.001$, main effect of genotype; $^{*}p < 0.05$, $^{**}p < 0.01$, genotype × treatment interaction (Bonferroni *post hoc*).

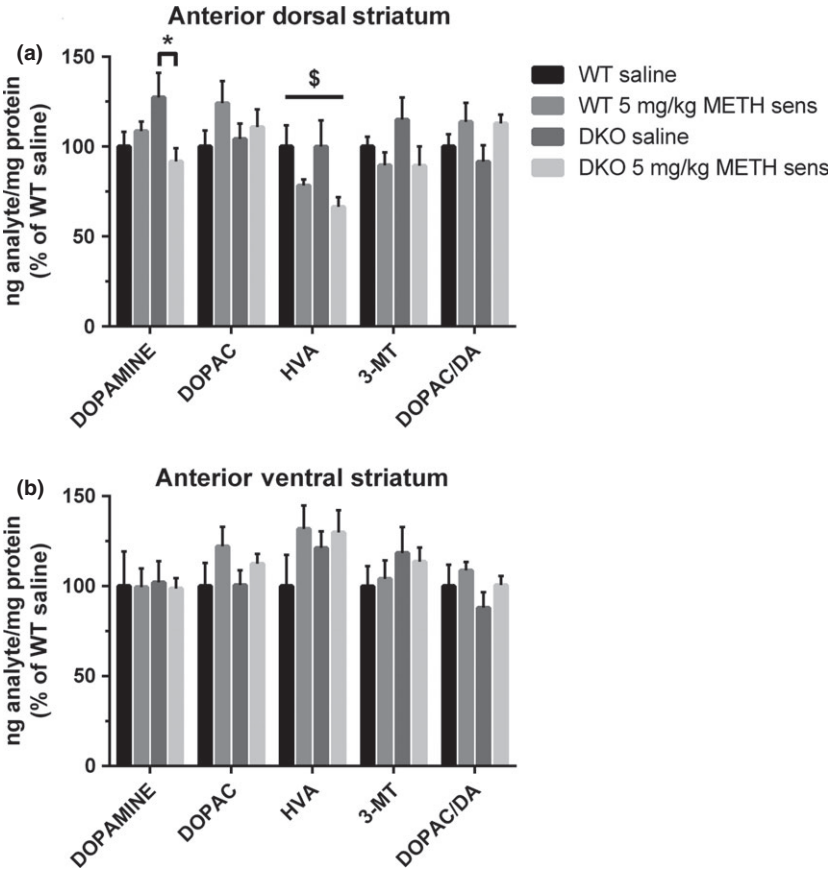

Fig. 4 Sensitized tissue content levels of dopamine and its metabolites measured in the (a) anterior dorsal striatum and (b) anterior ventral striatum (NAc) of wild-type mice (WT) and DKO mice (n = 4–8/group). Tissues were collected 24 h following a challenge injection of saline or methamphetamine (METH, 5 mg/kg) on day 13 of the locomotor sensitization procedure. Data reported as average percentage change over WT saline-treated controls and independent two-way ANOVA analysis was conducted for each analyte. $^{\$}p > 0.05$, main effect of METH treatment; $^{*}p < 0.05$, genotype × treatment interaction (Bonferroni *post hoc*).

injection of 5 mg/kg METH (i.p., day 13) in the locomotor sensitization procedure (Fig. 4a and b, respectively).

Within the acute METH-treated cohort, independent two-way ANOVA analyses revealed a main effect of genotype for dopamine [$F(1, 28) = 14.41$, $p < 0.001$] and its main intracellular metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) [$F(1, 28) = 4.05$, $p = 0.054$] in the dorsal striatum (Fig. 3a), which were elevated in saline-treated DKO mice compared to WT controls. A significant genotype × treatment interaction was also detected in this brain region for the extracellular dopamine metabolite homovanillic acid (HVA) [$F(1, 28) = 8.65$, $p < 0.01$] (Fig. 3a). *Post hoc* analysis showed a genotypic enhancement in HVA levels in saline-treated DKO versus WT controls ($p < 0.01$). Furthermore, HVA levels were divergently modulated by acute METH, in that they were increased in METH-treated WT mice but markedly decreased in METH-treated DKO mice ($p < 0.05$). While a significant main effect of METH treatment was also observed in HVA levels measured in the ventral striatum [$F(1, 25) = 8.96$, $p < 0.01$] (Fig. 3b), METH exposure conversely reduced HVA content in both WT and DKO-treated groups relative to saline controls. Analysis of both the dorsal (Fig. 3a) and ventral (Fig. 3b) striatum revealed significant main effects of METH administration on DOPAC levels [$F(1, 28) = 4.79$, $p < 0.05$ and $F(1, 25) = 14.32$, $p < 0.001$, respectively] and DOPAC to dopamine turnover ratios [$F(1, 28) = 12.70$, $p < 0.01$ and $F(1, 25) = 14.48$, $p < 0.001$, respectively]. Reductions in DOPAC content were observed in both brain regions and genotypes following METH, leading to a decrease in the DOPAC to dopamine ratio in all METH-treated groups.

In sensitized mice, significant alterations in dopamine and its metabolite, HVA, were detected in tissues collected 24 h after a 5 mg/kg METH challenge within the dorsal (Fig. 4a) but not the ventral striatum (Fig. 4b). Two-way ANOVA analysis showed a significant genotype X treatment interaction [$F(1, 27) = 5.31$, $p < 0.05$] in dorsal striatal dopamine content (Fig. 4a). *Post hoc* comparison revealed dopamine levels were significantly attenuated in METH-sensitized DKO mice, but not WT mice, relative to respective saline-treated controls ($p < 0.05$). A main effect of treatment was also observed for HVA levels in the dorsal striatum [$F(1, 27) = 7.77$, $p < 0.05$] (Fig. 4a), which were measurably decreased in both WT and DKO mice sensitized to METH compared to saline-treated controls.

Aberrant modulation of the striatal post-synaptic regulator protein DARPP-32 in mice lacking AC 1/8

DARPP-32 is a critical downstream effector of post-synaptic PKA signaling underlying the behavioral sensitization response to stimulants (Lin *et al.* 2002; Chen and Chen

2005; Valjent *et al.* 2005; Zachariou *et al.* 2006; Scheggi *et al.* 2007). To establish if the attenuated striatal dopamine content and locomotor sensitization response to METH in DKO mice was associated with impaired activation of DARPP-32, levels of phosphorylated Thr-34 and Thr-75, as well as total DARPP-32 and actin (as control), were assessed using immunoblot analysis from dorsal striatal tissue collected 24 h after a challenge injection of saline, 1, or 5 mg/kg METH on day 13 of the sensitization procedure in WT and DKO mice (Fig. 5a).

Assessment of DARPP-32 phosphorylation at Thr-34 following sensitization revealed a significant main effect for both genotype [$F(1, 17) = 5.248$, $p < 0.05$] and treatment [$F(2, 17) = 3.799$, $p < 0.05$] (Fig. 5b). Whereas METH sensitization enhanced the level of pThr-34-DARPP-32 in WT mice in a dose-dependent manner, this effect was blunted in DKO mice that displayed an elevated basal level of Thr-34 phosphorylation compared to saline-treated WT mice. Evaluation of the phosphorylation state of the other major regulatory site of DARPP-32, Thr-75, showed a significant genotype x treatment interaction [$F(2, 18) = 8.128$, $p < 0.01$] (Fig. 5c). *Post hoc* analysis indicated that the Thr-75 site of DARPP-32 was also hyperphosphorylated in saline-treated DKO mice relative to WT saline controls ($p < 0.001$). Whereas METH sensitization induced a slight increase in Thr-75 phosphorylation of DARPP-32 in WT mice, similar treatment in DKO mice resulted in a reduction in pThr-75-DARPP-32 levels. No effect of genotype or treatment was observed in the levels of total DARPP-32 in the dorsal striatum (Fig. 5d). As the activity state of DARPP-32 is regulated by the balance of phosphorylation at these residues, the ratio of Thr-34 to Thr-75 phosphorylation was analyzed and indicated a significant main effect of genotype [$F(1, 15) = 6.96$, $p < 0.05$] and treatment [$F(2, 15) = 5.99$, $p < 0.05$] (Fig. 5e). DARPP-32 is predominately phosphorylated at Thr-75 under basal conditions (Svenningsson *et al.* 2004), which was presently observed in the dorsal striatum of saline-treated WT mice. In saline-treated DKO mice, the level of DARPP-32 phosphorylation at both the Thr-34 and Thr-75 sites was increased relative to saline-treated WT controls, resulting in an equal phosphorylation ratio at these residues. Repeated exposure to METH in the sensitization procedure enhanced the phosphorylation of DARPP-32 at Thr-34 relative to Thr-75, an effect that was attenuated in DKO mice relative to WT mice.

Discussion

This study presents key evidence that the calcium/calmodulin-stimulated AC enzymes, AC 1 and AC 8, contribute to

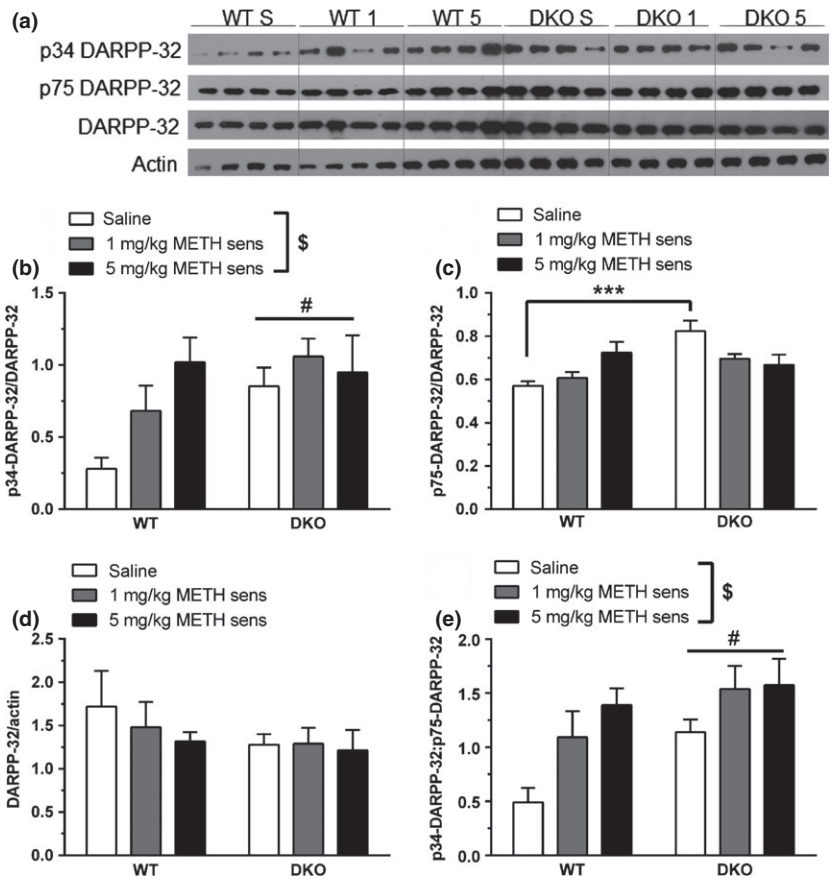

Fig. 5 (a) Representative immunoblots of DARPP-32 in the anterior dorsal striatum of wild-type mice (WT) and DKO mice assessed 24 h following a challenge injection of saline (S) or methamphetamine (METH) on day 13 of the sensitization procedure. Each lane is representative of an individual mouse ($n = 4$/group). Bar graphs illustrate quantitative measurement of either (b) pThr-34-DARPP-32 or (c) pThr-75-DARPP-32 normalized to total DARPP-32, (d) total DARPP-32 normalized to actin, or (e) ratio of pThr-34-DARPP-32 to pThr-75-DARPP-32 calculated from westerns run with two independent sets of mouse brain samples for each group (total of $n = 8$/group). Each data set was independently compared by two-way ANOVA analysis. $^{\$}p < 0.05$, main effect of treatment; $^{\#}p < 0.05$, main effect of genotype; $^{***}p < 0.001$, genotype × treatment interaction (Bonferroni *post hoc*).

METH-induced behavioral responses and accompanying METH-mediated changes in pre-and post-synaptic striatal dopamine function. Evaluation of DKO mice that have a genetic deletion of AC 1/8 revealed a heightened behavioral and neuronal responsivity to dopamine in the dorsal striatum in saline-treated animals. This finding indicates a previously unidentified role for AC 1/8 in maintaining basal dopamine function, which may alter the sensitivity of the nigrostriatal pathway to METH exposure. Congruently, DKO mice displayed an attenuated acute locomotor response to METH, which coincided with a reduction in HVA, reflective of decreased extracellular dopamine levels and altered dopamine metabolism in the dorsal, but not ventral striatum. Upon repeated METH exposure, DKO mice showed a blunted locomotor sensitized response, as well as a long-lasting reduction in dopamine tissue content and deficits in post-synaptic DARPP-32 activation in the dorsal striatum. Together, these observations indicate calcium-stimulated ACs mediate the stimulatory and neuroplastic effects of METH by regulating the efficacy of drug-induced recruitment of the dorsal striatal dopaminergic system.

Basal state changes

A novel finding that stemmed from the present study of the effects of calcium-stimulated ACs on METH revealed a neuromodulatory role for these AC isoforms on basal state dopaminergic function in the striatum. To date, the neuronal effects of AC 1/8 have mainly been defined in the hippocampus and cortex, which indicate they functionally contribute to pre-and post-synaptic long-term potentiation (Villacres *et al.* 1998; Wong *et al.* 1999), are required for PKA-mediated regulation of pre-synaptic vesicular release proteins (Conti *et al.* 2009a), and mediate restoration of pre-synaptic function following strong neuronal activity (Moulder *et al.* 2008). In this study, DKO mice showed up-regulated tissue levels of all dopaminergic markers in the dorsal striatum with statistically significant elevations in dopamine, DOPAC, and HVA content. Dopamine released into the synaptic cleft can be metabolized by MAO or catechol-*O*-methyl-transferase (COMT) in surrounding glial cells. HVA is one of the main dopamine degradation products that results from 3-*O*-methylation of DOPAC by COMT. As there is no COMT activity in dopaminergic neurons, the heightened HVA levels presently observed in DKO mice likely reflect increased dopamine overflow. Correspondingly, elevations in HVA levels have been correlated clinically to a hyperactive phenotype (Castellanos *et al.* 1994, 1996), which we and others observed behaviorally in DKO mice (Wieczorek *et al.* 2010; Conti *et al.* 2012). Increased dopaminergic tone in the dorsal striatum would also coincide with the presently observed up-regulated basal levels of DARPP-32 phosphorylation in this region as a consequence of enhanced activation of post-synaptic dopamine receptors on striatonigral and striatopallidal neurons (Bateup *et al.* 2008). In particular, activation of D1 dopamine receptors *in vivo* results in a large potentiation of pThr-34 levels in both neuronal populations and a slight increase in pThr-75 in striatopallidal neurons (Bateup *et al.* 2008), which may explain the shift toward an equal basal phosphorylation ratio of these DARPP-32 residues in DKO mice. Up-regulated basal levels of DARPP-32 phosphorylation, particularly at Thr-34, are also associated with a phenotypic expression of hyperactivity (Beaulieu *et al.* 2006), which is consistent and perhaps points to a mechanism underlying the heighted locomotor activity in DKO mice. Future dissection of the cell type-specific changes in striatonigral and striatopallidal signaling known to differentially regulate striatal motor activity (Bateup *et al.* 2010), would provide critical information to the mechanism underlying the hyper-locomotor phenotype of DKO mice.

The modulatory effect of AC 1/8 on basal dopamine function appeared to be confined to the dorsal aspect of the striatum, as no changes in dialysis dopamine levels or tissue dopamine and metabolite content in the NAc/ventral striatum were detected in DKO mice. This specificity corresponds with the predominance of at least AC 1 expression in this region relative to the ventral striatum (Yao *et al.* 2004; Olsen *et al.* 2008). Also of note, basal dopamine system changes were only detected in tissues from DKO mice collected 30 min after acute saline exposure (day 5, Fig. 3) and not from tissue collected 24 h after repeated saline treatment (day 13, Fig. 4). As mice deficient in calcium-stimulated ACs display abnormal stress adaptation and reactivity (Schaefer *et al.* 2000; Razzoli *et al.* 2010; Wieczorek *et al.* 2012), it must be considered that this finding could reflect an altered neurochemical response to handling/injection stress that may diminish with time or following daily handling in the sensitization procedure. Future investigation into the synaptic targets and role of COMT activity changes that may underlie the disrupted striatal dopamine homeostasis in AC 1/8-deficient mice are warranted to test these hypotheses. Although the focus of this study is to elucidate the role of calcium-stimulated ACs in drug-induced plasticity, this research has broad implications for aberrant AC 1/8 signaling in other co-morbid psychiatric disorders, such as post-traumatic stress disorder and schizophrenia, which are associated with persistent hyperdopaminergic abnormalities (Auxemery 2012; Snyder and Vanover 2014).

Altered acute response to METH

Prior reports indicate that locomotor responses to abused substances were affected by loss of calcium-stimulated ACs, with DKO mice demonstrating an increase in cocaine-induced (DiRocco *et al.* 2009) and a decrease in ethanol-induced locomotor stimulation (Maas *et al.* 2005; Conti *et al.* 2009a, 2012). In this study, absence of AC 1/8 in DKO mice resulted in reduced locomotor responsivity to acute METH stimulation. Enhanced striatal dopamine transmission

is generally thought to contribute to the locomotor stimulatory properties of these drugs. METH promotes extraneuronal dopamine increases through multi-faceted pre-synaptic effects that reduce uptake and increase efflux through DAT, inhibit vesicular monoamine transporter activity, and suppress MAO intracellular catabolism of dopamine to DOPAC (Kelly and Iversen 1976; Fleckenstein *et al.* 2007; Fornai *et al.* 2009; Brabant *et al.* 2014). While DKO mice did not display altered METH (5 mg/kg)-induced NAc dialysis dopamine efflux or reductions in striatal DOPAC catabolism, a genotypic difference in the levels of HVA was observed within the dorsal striatum. In METH-treated WT mice, the levels of HVA and 3-methoxytyramine (3-MT) increased, reflecting enhanced extracellular availability of dopamine that was not present in DKO mice. Rather, the significant reduction in HVA levels following METH suggests DKO mice have a deficit in METH-elicited increases in extracellular dopamine that would correspond with their blunted acute locomotor stimulatory response to METH. The present finding that dysregulated basal dopamine function in DKO mice was also isolated to the dorsal striatal subregion indicates these changes may have impacted METH-induced activation of this system. Moreover, these observations suggest calcium-stimulated ACs modulate locomotor activity through regulation of pre-synaptic nigrostriatal dopaminergic transmission, which may explain why the absence of AC 1/8 divergently impacts the locomotor effects of drugs that act at different pre-synaptic sites of action (Sulzer *et al.* 2005; Fleckenstein *et al.* 2007).

Impaired plasticity related to METH sensitization

AC 1/8 are known to integrate PKA-mediated neuroplastic events that regulate synaptic efficacy, long-term potentiation and associated forms of hippocampal-dependent learning and memory (Xia and Storm 1997; Wang and Storm 2003; Zhang *et al.* 2008, 2011). As such, this study aimed to identify a role for AC 1/8 in the neuroplastic response to METH using behavioral sensitization, which involves cAMP/PKA signaling. Both WT and DKO mice displayed a dose-dependent increase in locomotor activity following sensitized exposure to METH relative to their acute response. However, DKO mice showed a significant attenuation of this response and a decreased expression upon challenge at both doses tested compared to sensitized WT mice. While METH can produce a dose-dependent shift from ambulatory to stereotypy behavior (Robinson and Becker 1986; Seiden *et al.* 1993; Saal *et al.* 2003), as presently observed with 5 mg/kg METH exposure, this effect was similar across genotypes and does not indicate that an increase in stereotyped behavior interfered with locomotor activity in DKO mice. Rather, the blunted sensitized response to METH in DKO mice implicates calcium-stimulated ACs contribute to the cAMP/PKA-mediated pathways known to play a prominent role in the enduring neuroadaptations recruited by METH to elicit behavioral sensitization (Moriguchi *et al.* 2002; Miyazaki *et al.* 2013) and are consistent with the observed changes in sensitized locomotor responding to cocaine reported in DKO mice (DiRocco *et al.* 2009). Interestingly, whereas METH in this study still elicited a sensitization response that was blunted compared to WT mice, this effect was completely absent following cocaine administration (DiRocco *et al.* 2009). This difference could reflect the interaction of AC 1/8 with distinct neuronal targets or differential efficacy of these drugs on neurotransmitter systems that warrant further investigation.

METH-induced locomotor sensitization is known to be mediated by neuroadaptations in striatal dopamine function, which appear to be dependent on dose and time point of neurochemical assessment. While the neurotoxic effects of repeated high-dose METH exposure (10–100 mg/kg acutely or binge administration of multiple 4–10 mg/kg injections during a daily session) to induce dopamine depletion are well-characterized [for review see (Kuhn *et al.* 2011; Carvalho *et al.* 2012; Panenka *et al.* 2013; Halpin *et al.* 2014)], relatively little is known regarding the functional alterations in dopamine content and metabolism following a low-dose METH sensitization procedure. The present assessment of these parameters in sensitized mice 24 h after a METH challenge (5 mg/kg) revealed a reduction in dorsal striatal HVA levels in both WT and DKO mice and a significant decrease in dopamine levels only in DKO mice. Transient decreases in dialysate dopamine levels, corresponding to augmented DAT expression and function, have been shown in the ventral striatum at the same time point (24 h) following chronic low-dose METH treatment (Broom and Yamamoto 2005). This finding is consistent with METH-induced changes in dopamine extracellular metabolites (i.e., a significant reduction in HVA and a similar trend in 3-MT levels) shown here in the dorsal striatum, but, is likely not related to the differences in behavioral sensitization between WT and DKO mice as these effects were observed in both genotypes. Sensitizing doses of METH have not previously been reported to alter dopamine tissue content, as seen presently in WT mice, but did reduce DOPAC to dopamine turnover (Kitanaka *et al.* 2003). In contrast to Kitanaka *et al.* (2003) and our acute study that detected reductions in DOPAC catabolism shortly after METH exposure (30 min), no effect of METH on DOPAC content in either striatal region was evident 24 h after exposure, indicating this may be a short-term compensatory mechanism. The pronounced reduction in dopamine levels following METH sensitization only observed in DKO mice correlates with their blunted locomotor sensitization, as it indicates a depleted supply of dopamine that is integral to trigger downstream effectors to generate this response. Further, as diminished dopamine levels are generally associated with neurotoxic regimens of METH exposure (Kuhn *et al.* 2011; Carvalho *et al.* 2012), this finding suggests mice lacking AC 1/8 may be more

susceptible to METH-induced striatal dopamine neurodegeneration. Similarly, DKO mice had increased vulnerability to striatal apoptosis following neonatal high-dose ethanol exposure, which was accompanied by suppression of phosphatidylinositol-3 kinase/Akt and ERK pro-survival pathways (Conti *et al.* 2009b). Notably, these pathways are also activated in response to METH neurotoxicity (Bourque *et al.* 2012) and previous evidence indicates that DKO mice had impaired activation of ERK signaling following cocaine (DiRocco *et al.* 2009).

To determine if dopamine deficits and reduced locomotor responding following METH sensitization in DKO mice were accompanied by impaired activation of dopamine-mediated post-synaptic signaling cascades, this study assessed the phosphorylation state of DARPP-32. DARPP-32 plays a critical role in the behavioral and biochemical actions of dopamine stimulating drugs that, in turn, regulate DARPP-32 activity through phosphorylation at Thr-34 and Thr-75 (Valjent *et al.* 2005; Zachariou *et al.* 2006; Scheggi *et al.* 2007). Both sites were shown to be required to obtain a normal locomotor sensitization response to cocaine (Zachariou *et al.* 2006), indicating it is vital for dopamine-mediated activation of associated downstream neuroadaptations. In particular, phosphorylation of DARPP-32 at the PKA regulated Thr-34 site is known to be an important mediator of psychostimulant-induced ERK activation in the striatum (Valjent *et al.* 2005). The finding that loss of AC 1/8 suppressed cocaine-evoked activation of the ERK pathway (DiRocco *et al.* 2009), suggests the ability of psychostimulant drugs to amplify the actions of dopamine D1 receptor/cAMP/PKA on downstream effectors through recruitment of DARPP-32 is impaired in DKO mice. In line with these observations, sensitized exposure to METH in this study induced a dose-dependent increase in dorsal striatal PKA/phosphor-Thr-32 DARPP-32 signaling, leading to an enhanced ratio of Thr-34 to Thr-75 phosphorylated DARPP-32 in WT mice, but not DKO mice. While it remains unclear if the presently identified basal hyperphosphorylation of DARPP-32 in DKO mice could mask an increase in this response evoked by METH owing to a ceiling effect, the functional implications of aberrant DARPP-32 regulation on METH-induced plasticity remain relevant. Furthermore, the effect of METH sensitization on DARPP-32 phosphorylation in this study was assessed 24 h after the challenge exposure, suggesting DKO mice have a general suppression of the enduring drug-induced neuroadaptations that would correspond to the observed deficits in sensitization behavior.

While our data reveal a novel regulatory role for calcium-stimulated ACs in tonic and drug-stimulated DARPP-32 signaling, future studies are warranted to dissect if AC 1/8 deficiency impacts the activation state of DARPP-32 through local alterations of post-synaptic signaling pathways or upstream modulation of dopamine release. The increased basal dopaminergic tone and decreased dopamine content following METH in DKO mice corresponded with basal and METH-induced changes in DARPP-32 phosphorylation state and locomotor activity, indicating that AC 1/8 modulate these outcomes through regulating the level of dopamine stimulation. However, observations from our lab indicate calcium-stimulated ACs, particularly AC 1, are more densely localized to post-synaptic cells within forebrain regions (Conti *et al.* 2007), including the striatum (unpublished observations), where they could locally modulate DARPP-32 activity. DARPP-32 activation is regulated by cAMP through dopamine D1/D2 receptor stimulation (Bateup *et al.* 2008), and calcium generated through glutamate activation of NMDA receptors (Halpain *et al.* 1990; Nishi *et al.* 2002). Integration of coincident dopamine and glutamate signals promotes striatal synaptic plasticity and sensitization behavior (Valjent *et al.* 2005; Scheggi *et al.* 2007). Stimulation of post-synaptic dopamine receptors likely regulates cAMP signals in MSNs through G-protein modulation of AC 5 (Herve 2011), which is inhibited by calcium (Guillou *et al.* 1999). As AC 1/8 are directly stimulated by calcium/calmodulin and reside in close synaptic proximity to NMDA receptors (Mons *et al.* 1995), they could function as downstream effectors of NMDA-mediated calcium responses and couple its activation to the cAMP signaling pathway (Cooper *et al.* 1995). In this way, AC 1/8 would amplify cAMP signals and PKA activation that regulate synaptic efficacy of corticostriatal synapses (Nakano *et al.* 2010). Indeed, the presence of calcium/calmodulin was shown to enhance the efficacy of dopamine to stimulate AC activity (Ahlijanian and Cooper 1988). Thus, AC 1/8 isoforms could act as coincidence detectors of convergent dopamine and glutamate signals controlling striatal function and psychostimulant-induced behavioral sensitization (Vanderschuren and Kalivas 2000; Kalivas 2009; Schmidt and Pierce 2010).

Conclusions

Neuroadaptations that contribute to METH synaptic plasticity in the striatum, and ultimately addicted behavior, involve cAMP signaling pathways. Recruitment of AC enzymes with different regulatory characteristics may confer an important level of specificity to drug-induced cAMP signals. Our study reveals loss of AC 1 and AC 8 isoforms, which uniquely couple activity-dependent increases in intracellular calcium to cAMP/PKA pathways, leads to enhanced dorsal striatal dopaminergic tone and disrupts METH-induced regulation of dopamine levels and activation of DARPP-32 mediating locomotor sensitization behavior. Using genome-wide expression profiling, AC 1 was one of the few genes to be identified as up-regulated in several genetic and pharmacological models of behavioral supersensitivity to psychostimulants and dopamine (Yao *et al.* 2004). In addition to suggesting future studies to identify the individual functional

contribution of AC 1 specifically, this finding lends general support to the postulate that calcium-stimulated ACs are a core feature of the persistent cellular modifications underlying behavioral plasticity. Thus, targeted manipulation of these enzymes, in particular AC 1, may impair drug-induced maladaptive changes in limbic circuitry that would provide therapeutic utility to ameliorate addictive behavior.

Acknowledgments and conflict of interest disclosure

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All experiments were conducted in compliance with the ARRIVE guidelines.

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