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Methamphetamine decreases k+ channel function in human fetal astrocytes by activating the trace amine-associated receptor type-1

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Abstract

Methamphetamine (Meth) is a potent and commonly abused psychostimulant. Meth alters neuron and astrocyte activity; yet the underlying mechanism(s) is not fully understood. Here we assessed the impact of acute Meth on human fetal astrocytes (HFAs) using whole-cell patch-clamping. We found that HFAs displayed a large voltage-gated K^+ efflux (I_{Kv}) through K_v/K_v- like channels during membrane depolarization, and a smaller K^+ influx (I_{kir}) *via* inward-rectifying K_{ir}/K_{ir}-like channels during membrane hyperpolarization. Meth at a 'recreational' (20 μM) or toxic/fatal (100 μM) concentration depolarized resting membrane potential (RMP) and suppressed $I_{Kv/Kv\text{-}like}$. These changes were associated with a decreased time constant (T), and mimicked by blocking the two-pore domain K^+ (K_{2P})/K_{2P}- like and K_v/K_v-like channels, respectively. Meth also diminished $I_{Kir/Kir\text{-}like}$, but only at toxic/fatal levels. Given that Meth is a potent agonist for the trace amine-associated receptor type-1 (TAAR1), and TAAR1-coupled cAMP/cAMP-activated protein kinase (PKA) cascade, we further evaluated whether the Meth impact on K^+ efflux was mediated by this pathway. We found that antagonizing TAAR1 with *N*-(3-Ethoxyphenyl)-4-(1-pyrrolidinyl)-3-(trifluoromethyl)benzamide (EPPTB) reversed Meth-induced suppression of $I_{Kv/Kv\text{-}like}$; and inhibiting PKA activity by H89 abolished Meth effects on suppressing $I_{Kv/Kv\text{-}like}$. Antagonizing TAAR1 might also attenuate Meth-induced RMP depolarization. Voltage-gated Ca^{2+} currents were not detected in HFAs. These novel findings demonstrate that Meth suppresses $I_{Kv/Kv\text{-}like}$ by facilitating the TAAR1/G_s/cAMP/PKA cascade and altering the kinetics of K_v/K_v-like channel gating, but reduces K_{2P}/K_{2P}-like channel activity through other pathway(s), in HFAs. Given that Meth-induced decrease in astrocytic K^+ efflux through K_{2P}/K_{2P}-like and K_v/K_v-like channels reduces extracellular K^+ levels, such reduction could consequently contribute to a decreased excitability of surrounding neurons.

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Keywords

astrocyte; calcium channel; electrophysiology; methamphetamine; potassium channel; TAAR1

Methamphetamine (Meth) is a psychostimulant that is highly addictive and widely abused in the USA (Panenka *et al.* 2013). Meth alters cognition and motivation-driven behaviors in humans, which are associated with neurodegeneration, microgliosis, and astrocytosis (Cadet and Krasnova 2009; Krasnova and Cadet 2009; Parsegian *et al.* 2011). Animal studies also indicate that Meth self-administration (Meth-SA) decreases neuronal excitability in the nucleus accumbens (NAc) (Graves *et al.* 2015) and medial prefrontal cortex (Chen et al. 2012), two major regulators of cognition and addiction. Other studies suggest that Meth-induced neuronal dysregulation is functionally affected by glia (Narita *et al.* 2006; Zhang *et al.* 2006; Beardsley *et al.* 2010; Fujita *et al.* 2012; Snider *et al.* 2012, 2013). However, little is known about the mechanism(s) by which Meth suppresses neuronal firing; and how Meth, besides disturbing catecholamine neurotransmission and glutamate uptake, alters astrocyte function (Cisneros and Ghorpade 2014), thereby altering excitability of surrounding neurons.

Astrocytes play a crucial role in the CNS, including, but not limited to, maintenance of extracellular levels of glutamate and K^+ ($[K^+]_o$) (Beardsley and Hauser 2014; Cheung *et al.* 2015; Kadala *et al.* 2015). Previous studies reveal that astrocytes functionally express various K^+ channel subtypes; while Meth alters neuronal and nonneuronal cell activity by altering K^+ channels. For example, Meth decreases the expression of alternatively spliced variants of Ca^{2+}-activated bulk-conductance K^+ channels in human frontal cortex neurons (Tatro *et al.* 2013), and reduces K^+ currents through them in mouse neurons (Wang *et al.* 2013). Meth also suppresses the expression of voltagegated K^+ (K_v) channels and inwardly rectifying K^+ (K_{ir}) channels (Qu *et al.* 2014), thereby inhibiting I_{Kir} and I_{Kv}, respectively, in rat cardiac myocytes (Liang *et al.* 2010). Meth-SA also diminishes K^+ efflux *via* G protein-coupled inwardly rectifying K^+ channels in rat dopaminergic neurons (Sharpe *et al.* 2015). However, very little is known about how and to what extent Meth alters K^+ channel activity in astrocytes.

Besides dysregulating the catecholamine neurotransmission, Meth also disrupts activity of K^+ channels by affecting trace amine-associated receptor type-1 (TAAR1)-coupled signaling. Meth is a potent agonist for TAAR1 (Bunzow *et al.* 2001; Cotter *et al.* 2015), and expressed widely in neurons (Xie and Miller 2009; Miller 2011; Liberles 2015) and astrocytes (Cisneros and Ghorpade 2014). TAAR1 mediates dopamine neurotransmission and astrocyte glutamate uptake by facilitating the TAAR1/G_s/cAMP/cAMP-activated protein kinase (PKA) signaling (Jing and Li 2015). Meth binds to TAAR1, which promotes the G_s protein-coupled PKA signaling, and G_q protein-coupled phospholipase C/protein kinase C (PKC) signaling (Miller *et al.* 2005; Xie *et al.* 2007; Xie and Miller 2009; Lin *et al.* 2010; Panas *et al.* 2012). Meth-induced TAAR1 activation also increases intracellular cAMP levels in primary human astrocytes (Cisneros and Ghorpade 2014). PKA-induced phosphorylation reduces K_v channel activity (Dong and White 2003), while reduced PKA activity is associated with decreased I_{Kir}, in cortical pyramidal neurons (Dong *et al.* 2004). Moreover,

PKC-induced phosphorylation also inhibits activity of the two-pore K^+ (K_{2P}) channel that controls K^+ efflux at resting status to maintain RMP (Veale *et al.* 2007). PKC-induced phosphorylation also inhibits K_{ir} channel activity in cardiac cells (Scherer *et al.* 2016). Together, these findings strongly suggest that Meth alters astrocyte-mediated K^+ homeostasis by disturbing the TAAR1-mediated PKA and PKC signaling pathway.

Our recent studies have been focusing on how acute Meth *in vitro* dysregulates activity and signaling pathway(s) in human fetal astrocytes (HFAs) (Sharma *et al.* 2011; Yu *et al.* 2017a), additional to that mediated by PKA and PKC. Such Meth dysregulation also contributes to the mechanism(s) by which Meth alters neuronal activity, and exacerbates the neuropathogenesis induced by comorbid HIV infection (Al-Harthi 2012; Narasipura *et al.* 2012). To advance our understanding for the Meth impact on K^+ channel activity and its underlying mechanism(s) in HFAs, we performed electrophysiological studies using whole-cell voltage-clamp recording of HFAs following acute Meth exposure (20 and 100 μM) *in vitro*. We selected such concentrations based on that the blood levels of Meth in 'recreational' users are reported at 0.2–17 lM (Melega *et al.* 2007); while higher Meth blood levels (~ 58–400 μM) could be toxic/fatal (Takekawa *et al.* 2007; Kiely *et al.* 2009). These clinical studies provide us the opportunity to suggest and use such 'recreational' and 'fatal/toxic' doses of Meth in the present study. Furthermore, using various K^+ channel blockers, TAAR1 antagonist, and PKA inhibitor, we also evaluated the mechanism(s) by which Meth affects astrocyte function.

The present study not only determines the acute effects of Meth *in vitro* on HFAs, but also defines a novel mechanism, by which acute Meth exposure alters astrocyte activity through altering K^+ channel function by interrupting the TAAR1-mediated signaling pathways. Importantly, that in turn could functionally disrupt astrocyte-neuron communication in the brain.

Materials and Methods

Cell culture and treatments

Primary HFAs (catalogue #CC-2565) were purchased from Lonza, Inc (Allendale, NJ, USA), and grown in clonetics astrocyte basal medium (CABM) supplemented with Astrocyte growth media Bullet-Kit (catalogue # CC-3186) (both from Lonza, Inc) in a 37°C incubator with 95% O_2 and 5% CO_2. HFAs (called Normal Human Astrocytes by Lonza) are not a cell line, which differ significantly from many cell lines. They are primary cells (not glioblastoma), and do not replicate. Using such HFAs, we avoided possible influences from other cells, especially those that can replicate and possibly take-over a dish. HFAs (up to the fourth splitting) were plated on petri dishes (~ 5000–8000 cells/dish) coated with polyornithine and fibronectin. Cells were fed with CABM 1 day after plating, and every 3–4 days thereafter. Prior to electrophysiological assessment, HFAs were treated in culture medium or bath with Meth, selective K^+ channel blockers, TAAR1 inhibitor, and/or compounds that affect related signaling (see *Selective blocker and inhibitor* section below). Cultured HFAs were used for electrophysiological studies within 2–5 days after plating.

In Meth studies, CABM containing Meth (20 or 100 μM) was used to treat cells acutely for 3–6 h prior to assessment. We chose this exposure time period based on the fact that peak plasma Meth concentrations are achieved in approximately 3–6 h after drug ingestion (Schep *et al.* 2010), during which brain astrocyte/neuron activity are altered. Our previous studies demonstrate that Meth exposure *in vitro* (10–1000 μM) for 5 days significantly alters HFA function, and repeated Meth administrations in 1 day also alter astrocyte function, and induces neuronal toxicity *in vivo* (Yu *et al.* 2017b). Our pilot data also showed that 20 μM Meth induced similar suppression on astrocytic K^+ currents after a 2-day exposure (data not shown) compared to a 3–6-h exposure. These findings encourage us to use 3–6-h Meth exposure regimen to evaluate acute effects of Meth on affecting functional activity of HFAs. Phosphate- buffered saline was used as a vehicle control. Before assessment, the media was replaced by the bath solution (see below in *Electrophysiology* section) containing the same concentration of Meth in pretreatment.

Electrophysiology

Whole-cell voltage-clamp mode was used for patch-clamping study. HFAs were visualized using a Nikon Diaphot 300 upright microscope (Nikon instruments, Melville, NY, USA). All HFAs were in isolation by visual inspection, in which neither the soma nor any process was physically contacted with any others. Healthy HFAs were chosen and studied using the following criteria: (i) had smooth edges with a more transparent appearance compared to dead cells; (ii) had a higher profile than the flat counterparts (that were extremely difficult to record); and (iii) HFAs were distinguished from neurons by their morphology and size in coculture. Specifically, HFAs had more than two processes and a flatter appearance, while Lund human mesencephalic neurons neurons were smaller (less than 17 pF). A Digidata 1440A digitizer and Axon 200B or 700A amplifier (Molecular Devices, Sunnyvale, CA, USA) were used for recording. Electrodes (4–10 MΩ) were pulled from borosilicate pipettes using a pipette puller P-97 (Sutter Instruments Co. Novato, CA, USA), and filled with pipette solution. The pipette solution contained (in mM): K-gluconate 120, HEPES 20, EGTA 0.1, KCl 10, $MgCl_2$ 1, Na_2ATP 4 (pH 7.29–7.31, 270–280 mOsm). For assessing the 'basal' condition, the bath solution contained (in mM): NaCl 135, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 5, KCl 4, glucose 5 (pH 7.37–7.43, 280–290 mOsm); but no ion channel blockers. For assessing the 'control' condition of K^+ channel activity, bath solution also contained blockers for the voltage-gated Na^+ channel (tetrodotoxin, TTX, 500 nM) (Abcam, Cambridge, MA, USA) and Ca^{2+} channel ($CdCl_2$, 2 μM) (Hu *et al.* 2004, 2005; Nasif *et al.* 2005a,b; Khodr *et al.* 2016).

For studying voltage-gated Ca^{2+} currents (VGCCs) in HFAs, all other non-Ca^{2+} ionic currents were blocked to ensure accurate evaluation of possible VGCCs in HFAs. In Ca^{2+} current (I_{Ca}) studies, the pipette solution contained (in mM): Cs-gluconate 140, HEPES 10, $MgCl_2$ 2, Na_2ATP, and NaGTP 0.3 (pH 7.29–7.31, 270–280 mOsm). Assessments were performed with blockade of (i) the voltage-gated Na^+ channels (TTX, 500 nM), (ii) voltage-gated chloride channels (4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid, 200 μM) (Tocris Biosciences, Bristol, UK) (Olsen *et al.* 2003), (iii) the hyperpolarization-activated (I_h) channels (2 mM CsCl), and (iv) various types of K^+ channels [using tetraethyl-ammonium (TEA), 20 mM]; and 4-aminopyridine (1–4 mM, a blocker for voltagesensitive A-type K_v

channels) (Calbiochem Inc, San Diego, CA, USA), and $BaCl_2$ (2 mM; this concentration blocked not only K_{ir} channels, but also K_v and K_{2P} channels) (Hille 2001; Hu *et al.* 2004, 2005; Nasif *et al.* 2005a,b; Khodr *et al.* 2016).

Extremely gentle suction (−0.1 to −1 psi) was applied to form a gig-ohm seal, aided with a Pyle PDMM 15 Manometer (Pyle Instruments, Brooklyn, NY, USA). The experimenter waited 1.5 to 3 min before breaking in to form whole-cell configuration. To allow time for solution equilibration, the first recording was performed at least 5 min after formation of the whole-cell configuration. Stable RMP was measured with the amplifier in $I = 0$ mode. For assessment of K^+ channel activities, the membrane potential (V_m) was initially held at −70 mV level, and currents were assessed from −50 to +100 mV with a 10 mV increment for 250 ms (unless specified). We chose this V_m range because we found that under our experimental condition, the reversal potential of HFAs was around −50 mV. For Ca^{2+} channel studies, voltages were tested in the range of −70 to +50 mV with 5 mV increments for 100 ms (cocultured), or 250 ms (cultured alone). Multiple recordings were performed and consistency was assessed.

pClamp 10 software (Molecular Devices) was used for acquisition and analysis. All the currents were measured at the steady state time point 10 ms prior to the end of each step. The current density (pA/pF) was compared *via* dividing the actual current magnitude by membrane capacitance (C_m). Input resistance (R_{in}) was calculated as 10 mV/ΔI, where ΔI = the difference between the steady state K^+ currents at the two voltage steps encompassing 0 pA. Given that moderately high series resistances were common (the mean of control cells was approximately 35 MΩ), Channellab software (Synaptosoft Inc, Atlanta, GA, USA) was used to correct the series resistance errors off-line and to achieve 100% correction (Traynelis 1998), while the correction and prediction in pClamp were set to zero during recording.

Ion blockers and inhibitors

All drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise specified. During recording, various ion channel blockers were added in bath solution for at least 10 min (unless specified). Quinidine (VWR, Radnor, PA, USA) is a blocker for a variety of K^+ channels, including human K_{2P} channel subtypes (TASK-1, TREK-1, and TREK-2) (Lotshaw 2007); and was used here to exam acute Meth effects on I_{K2p}, a 'leaking' K^+ efflux that maintains RMP and is mediated mainly by K_{2P} channels (Hille 2001). Given that K_{2P} channels are commonly expressed in astrocytes (Kettenmann and Ransom 2013), and the IC_{50} of quinidine was ~ 100 μM in blocking human K_{2P} channels (Lotshaw 2007), 1 mM quinidine was used to ensure its maximal effects on blocking I_{K2P}. Another K^+ blocker, TEA, was used to determine if it could mimic and confirm the direct, acute effects of Meth on suppressing voltage-sensitive K^+ channels during V_m depolarization in HFAs *in vitro*.

The TAAR1 antagonist EPPTB (25 nM) (Tocris Biosciences, Bristol, UK) was used to block or reverse acute Meth effects on TAAR1 (Bradaia *et al.* 2009). It is worth noting that EPPTB also acts as an *inverse agonist*, which could have an opposite effect on TAAR1 compared to a TAAR1 agonist (Bradaia *et al.* 2009). On the day of electrophysiological assessment, cells were pre-treated with CABM containing EPPTB for 30 min, followed by treatment with

CABM-containing EPPTB (25 nM) + Meth (20 μM) for 3–6 h, prior to recording. H89 (Tocris Biosciences, Bristol, UK), a PKA inhibitor (Lochner and Moolman 2006; Murray 2008), was also used to determine if Meth exerted its acute effects on K^+ channel activity through the cAMP/PKA cascade. During recordings, HFA currents were assessed prior to H89 application, and then were treated with H89 (10 μM) for 15–20 min before a further assessment.

Measurement of Time Constant

The time constant (T) was assessed at all V_m levels in HFAs to determine if there was any significant change in the kinetics of voltage-sensitive K^+ channel gating induced by Meth, which could be reflected partly by altered activation of K^+ currents through these channels. The time courses of I_{Kv} traces were fitted over a 100 ms time period using a two term exponential function (Levenburg–Marquardt search and Sum-of-Squares minimization method) in Clampfit 10.7. This measurement obtained two T: the fast T that reflects the kinetics of K^+ channel gating in the initial period of the activation prior to the steady state; and the slow T that reflects the kinetics of K^+ channel gating mainly in an early period of the steady state; and this would normally include an influence from the inactivation of some A-type K^+ channels (which are more quickly activated, and then inactivated, than other subtypes of K_v channel, a.k.a. the delayed rectifier). Individual points of the T were excluded if they did not fit properly (e.g., the regression fails to fit, or the value of the T was over 5000 ms). The outlier cells (which had two or more V_m levels excessing the ± twofold SD of the mean) were excluded (also see the Results section for more detail).

Statistical Analysis

Student's t-tests were used for the comparison of R_{in}, C_m, and RMP from basal control versus vehicle-treated control cells. One-way ANOVA was used for the comparison of RMP in K_{2P} channel studies. In this study, replication refers to the same cell before and after treatment or at different time points. Two-way ANOVA with repeated measurement (rm) was used to assess the effects of Meth, inhibitors or blockers on K^+ currents in HFAs, with the exception of the TEA and Meth effects which was analyzed first using three-way ANOVA. When there was no significant difference in the three-way interactions, but a significance difference in the main factors and/or two- way interaction, data analysis was further performed using the two- way rmANOVA. All ANOVA were followed by *post hoc* tests as specified. Cells were excluded if they were found to be the outliers (which had a value that exceeded the mean ± 2 × standard deviation, SD) in any category, including R_{in}, RMP, C_m, and series resistance. All values were reported as the mean standard error (SE). The 'n' in the figure legend indicates the number of cells. Statistical analysis was conducted accordingly using Prism 7 (GraphPad Software Inc., La Jolla, CA, USA) and considered significant when $p \leq 0.05$. Some raw data were used two or three times for statistical data analysis. Under such experimental conditions, the Bonferroni correction was applied and $\alpha = 0.025$ or 0.0167 (instead of 0.05) was used appropriately. For example, the RMP measurement from all the HFAs under the *control* condition ($n = 41$; e.g., with blockade of voltage-gated Ca^{2+} and Na^+ channels) were used to compare with the *basal* condition (without blockade of voltage-gated Ca^{2+} and Na^+ channels). Among which, a subset of control cells ($n = 37$) was used again for assessing the acute effects of Meth (with different

doses, Fig. 1c) on RMP and the effect of EPPTB on RMP (Fig. 1e); and the rest of that group of data (n = 4; Fig. 1d) were used to access the effects of Meth and quinidine on RMP. The data of K_v currents from the control cells and the Meth-treated cells were also used twice for assessing the effects of Meth (with different doses; Fig. 2), and the effects of Meth plus EPPTB (Fig. 4) on K_v currents. This study was not preregistered. No blinding, randomization, or sample size calculation was performed in this study.

Results

Electrophysiological properties of HFAs

We conducted voltage-clamp recordings to assess the membrane properties of cultured primary HFAs (Fig. 1a), including RMP, R_{in}, and C_m. We found that RMP was −42.0 ± 6.8 mV (n = 5, range of −27 to −63 mV) in HFAs at a basal condition (without blockade of any type of membrane ion channels). This result is consistent with previous studies of cultured astrocytes, showing a more depolarized RMP in immature astrocytes from the neocortex of prenatal rats (−22 to −82 mV) (McKhann *et al.* 1997), or in freshly isolated astrocytes from the hippocampus of postnatal rats (−25 to −85 mV) (Zhou and Kimelberg 2000) (also see more detail regarding this issue in the Discussion section). We also found that R_{in} was 181.5 ± 67.2 MΩ, and C_m was 18.9 ± 5.9 pF in HFAs (n = 5). There was no any type of voltage sensitive spike in HFAs in response to membrane depolarization (Fig. 1b).

We further assessed the membrane properties of HFAs, with or without blockade of voltage-gated Na^+ channels (with TTX, 500 nM) and Ca^{2+} channels (with $CdCl_2$, 2 μM). We found that Na^+ and Ca^{2+} channel blockade did not significantly affect the HFA membrane properties, including RMP (without blockers: n = 5, −42.0 ± 6.8 mV; with blockers: n = 41, −43.3 ± 1.3 mV; t_{44} = 0.287, p = 0.775), R_{in} (without blockers: n = 5, 181.5 ± 67.2 MΩ; with block-ers: n = 37, 275.4 ± 34.4 MΩ; t_{40} = 0.964, p = 0.341), and C_m (without blockers: n = 5, 18.9 ± 5.9 pF; with blockers n = 37, 31.2 ± 2.3 pF; t_{40} = 1.822, p = 0.076). Given that astrocytes play a critical role in K^+ buffering; and their RMP depends upon K^+ channel activity, these findings suggest that the activity of HFAs also relays on normal function of K^+ channels. Thus, the following experiments throughout the present study were conducted and focused on K^+ channel activity under blockade of voltage-sensitive Na^+/Ca^{2+} currents at RMP and during V_m changes.

Meth depolarized RMP of HFAs

RMP of cells is established by K^+ efflux at resting states (e.g., I_{K2P}), which is typically regulated by K_{2P} channels (Kim 2005), and some K_{2P}-like K_{ir} channels in mature astrocytes (Olsen *et al.* 2006; Olsen and Sontheimer 2008; Kettenmann and Ransom 2013). To determine Meth effects on RMP of HFAs, we assessed RMP that was exposed to 20 or 100 μM Meth *in vitro*. We found that both 20 μM Meth (n = 17, −35.6 ± 1.8 mV) that mimicked non-lethal blood levels of Meth in Meth users (Melega *et al.* 2007), and 100 μM Meth (n = 12, −37.4 ± 2.9 mV) that was similar lethal Meth levels (Takekawa *et al.* 2007; Kiely *et al.* 2009), induced a significant RMP depolarization in HFAs compared to those in the control condition (n = 37, −43.8 ± 1.4 mV; One-way ANOVA: $F(2,63)$ = 6.119,p = 0.004) (Fig. 1c). There was no significant difference between RMP depolarization induced by 20 or 100 μM

Meth, suggesting that 20 μM Meth exerted a maximal effect on suppressing I_{K2P}/$I_{K2P\text{-like}}$ that led to RMP depolarization in HFAs.

To determine whether Meth-induced RMP depolarization resulted from reduced activity of K_{2P}/K_{2P}-like channels, we assessed the effects of K_{2P}/K_{2P}-like channel blockade by quinidine (1 mM, a general blocker for various K^+ channels including human K_{2P} channels) on RMP (Lotshaw 2007); and then compared that with the effects of quinidine + Meth (20 μM) on RMP. We found that quinidine blockade also significantly depolarized RMP compared to HFAs in control condition. Combined quinidine + Meth treatment did not induce further depolarization compared to that induced by quinidine alone (n = 4/ea; Control: −38.2 ± 1.9 mV; quinidine: −12.7 ± 5.9 mV; quinidine + Meth: −14.0 ± 2.9 mV; One-way ANOVA: $F_{(2,9)}$ = 13.1, p = 0.002) (Fig. 1d). Together, these findings suggest that Meth depolarizes RMP by suppressing K_{2P} or K_{2P}-like channels that conduct outflowing K^+ at the rest.

We then determined whether Meth-induced RMP depolarization was mediated by TAAR1 using the TAAR1 antagonist EPPTB (25 nM; which also is an inverse agonist of TAAR1) (Bradaia *et al.* 2009), and H89 (10 μM), an inhibitor for PKA which is a downstream player in the TAAR1-mediated signaling pathway (Cisneros and Ghorpade 2014). We found that neither EPPTB (Control: n = 37, −43.8 ± 1.4 mV; Meth: n = 17, 35.6 ± 1.8 mV; Meth + EPPTB: n = 9, 38.5 ± 1.5 mV; One-way ANOVA: $F_{(2,60)}$ = 6.598, p = 0.003) (Fig. 1e), nor H89 (data not shown), blocked Meth-induced RMP depolarization (p > 0.05). Intriguingly, there was also no significant difference (p > 0.05) in RMP between control cells and those treated by Meth + TAAR1 antagonist (Fig. 1e). Collectively, these results suggest that, although acute Meth effect on depolarizing RMP appears to be PKA-independent, we can't rule out the possibility that Meth reduces K_{2P}/K_{2P}-like channel activity by activating TAAR1 through other mechanisms (e.g., the G_q/phospholipase C (PLC)/protein kinase C (PKC)/K_{2P} channel signaling pathway). Future investigation is needed to clarify this issue.

Meth reduced voltage-sensitive K^+ efflux (I_{Kv}) in HFAs

K_v channels (including, but not limited to, the delayed rectifier) play a key role in astrocyte activation (MacFarlane and Sontheimer 1997). By conducting K^+ efflux, K_v channels regulate the extracellular K^+ homeostasis (Somjen *et al.* 2008); and in-turn modulates neuronal activity. To examine the Meth effect (20 or 100 μM) on K_v/K_v-like channels (Olsen and Sontheimer 2008), we assessed I_{Kv}. We found that HFAs displayed a large voltage-dependent I_{Kv} (i.e., indicated by the upward traces) (Fig. 2a, Control). The current-voltage relationships (I-V curves) show that astrocytic I_{Kv} was activated by V_m depolarization from a holding potential V_h = −70 mV to −50 mV and above up to +100 mV 2b, Control). Given that other types of voltage-gated ion channels were blocked; and that the reversal potential (approximately −50 mV) for this K^+ efflux was near the V_m at which K_v channels were normally activated, this finding suggest that the evoked K^+ efflux (I_{Kv}) was mediated mainly by K_v channels.

We also found that acute exposure to Meth (20 μM, n = 19; and 100 μM, n = 9) induced a significant reduction in I_{Kv} compared to vehicle-treated controls (n = 22; Two- way rmANOVA: interaction, $F_{(30,705)}$ = 2.784, p < 0.001) (Fig. 2a and b). I_{Kv} was further

suppressed by 100 μM Meth compared to 20 μM Meth. The *X*-axis and the *Y*-axis indicate the V_m and current density (pA/pF), respectively. The different Y intersects in the *I-V* plots indicate different K^+ current densities measured at the point of zero voltage level under blockade of other voltage-sensitive ion channels. Given that the 'conventional' K_v channel (a.k.a. the delayed rectifier) is activated at V_m around −60 mV level, whether these K^+ currents out-flowing at V_m = 0 mV level are actually through such K_v channels, or *via* other K_v-like channel(s), needs to be identified in future studies.

We also assessed Meth effects on the *T* (which partly reflects the alterations in the kinetics of K_v/K_v-like channel gating). We found that the fast *T* was significantly decreased in HFAs following Meth treatment compared to controls (control $n = 20$, Meth $n = 14$, Two-way rmANOVA: treatment: $F_{(1,32)} = 5.377$, $p = 0.027$) (Fig. 2c). In contrast, there was no significant change in the slow *T* ($p > 0.05$). Together, these results suggest that, in a dose-dependent manner, Meth reduces I_{Kv} by suppressing K_v/K_v-like channel activity; and this I_{Kv} reduction is associated with alterations in the kinetics of K_v/K_v-like K^+ channel gating, including but might not be limited to, a significantly decreased fast *T* during an early period of the activation of K_v/K_v-like channels, but not in their steady state. Future investigations are needed to reveal whether and how the activation, and inactivation, of K_v/K_v- like channels in mature astrocytes are altered after chronic exposure to Meth *in vivo*.

Meth-induced I_{Kv} reduction was mimicked by blockade of K_V channels

To determine whether Meth-induced reduction of K^+ efflux during V_m depolarization was mediated by decreased K_v channel activity, we mimicked this Meth effect by blocking I_{Kv} with TEA (20 mM) (Fig. 3a). We found that blockade of K_v/K_v-like channel activity with TEA during V_m depolarization significantly reduced I_{Kv} in the control group ($n = 6$/ea.; Two-way rmANOVA: interaction: $F_{(15,75)} = 6.397$, $p < 0.001$) (Fig. 3b). This TEA-induced I_{Kv} reduction was similar to that induced by Meth ($n = 4$) (Fig. 3b). Combined Meth + TEA treatment ($n = 4$) induced a further reduction in I_{Kv} compared to either one alone (Two-way rmANOVA: Meth vs. Meth + TEA: interaction: $F_{(15,45)} = 4.308$, $p < 0.001$; TEA vs. Meth + TEA: interaction: $F_{(15,120)} = 3.027$, $p < 0.001$) (Fig. 3b). These results indicate that Meth suppresses astrocytic I_{Kv}, in part by reducing K_v channel activity. However, combined Meth/TEA treatment induced a significantly greater decrease in I_{Kv}. Although the mechanism underlying this additional decrease is unknown, this result suggests that Meth and TEA might affect different subtypes of K_v (or K_v-like) channels.

Antagonism of TAAR1 abolished the acute effects of Meth on suppressing I_{Kv} in HFAs

To determine if Meth suppressed I_{Kv} by activating TAAR1 in HFAs, we antagonized TAAR1 with EPPTB, and assessed I_{Kv} that was activated by V_m depolarization from −50 mV to +100 mV in HFAs treated with vehicle (control, $n = 22$), Meth (20 μM, $n = 19$), or combined Meth (20 μM) + EPPTB (25 nM, $n = 9$) (Fig. 4a). We found that Meth decreased I_{Kv}, but combined EPPTB/Meth treatment completely reversed Meth-induced suppression of I_{Kv} in HFAs (Two-way rmANOVA: interaction: $F_{(30,705)} = 2.112$, $p < 0.001$) (Fig. 4b). Antagonizing TAAR1s by EPPTB alone did not induce any significant change in I_{Kv} compared to control HFAs or Meth + EPPTB; and there was no significant difference in the I_{Kv} density between EPPTB + Meth and EPPTB alone (both $p > 0.05$; data not shown). Together, these findings

suggest that Meth reduces I_{Kv} by suppressing K_v channel activity; and that is mediated by TAAR1-coupled signaling in HFAs.

Inhibition of PKA-like protein kinases abolished acute Meth effects on suppressing I_{Kv} in HFAs

PKA is a downstream substrate in the TAAR1-mediated signaling pathway (Cisneros and Ghorpade 2014). To evaluate the role of PKA-like protein kinases on the dynamic activity of K_v channels in HFAs, we assessed the effects of H89, a PKA inhibitor, on Meth-induced decrease of I_{Kv}. We found that Meth-induced suppression of I_{Kv} was completely abolished by concurrent application of H89 plus Meth (n = 5/ea; Two-way rmANOVA: interaction: $F_{(30,180)} = 2.178$, $p < 0.001$) (Fig. 5a and b). There was no significant difference in the I_{Kv} density between H89 + Meth and H89 alone ($p > 0.05$; data not shown). These results suggest that inhibition of PKA-related protein kinases blocks the acute Meth effects on suppressing I_{Kv} *via* K_v/K_v-like K^+ channels in HFAs, which is mediated by facilitating the TAAR1-mediated cAMP/PKA cascading.

HFAs displayed inwardly rectifying K^+ influx (I_{Kir}) in response to V_m hyperpolarization; and that was not affected by lower level of Meth

In contrast to a large I_{Kv}, we found that HFAs displayed a small I_{Kir}, which was mainly mediated by activated K_{ir} channels in response to V_m hyperpolarization (V_m = −60 to −140 mV levels; the downward traces in Fig. 1b). These K_{ir} channels were activated at V_m levels more hyperpolarized than −50 mV that induced K^+ *influx*. Typically they are expressed at very low levels in immature astrocytes as reported by previous studies (Kressin *et al.* 1995; Olsen *et al.* 2006; Olsen and Sontheimer 2008; Montiel-Herrera and Garcia-Colunga 2010). Thus, I_{Kir} was relatively small compared to I_{Kv} during V_m depolarization (the upward traces in Fig. 1b). Previous studies show that Meth decreases I_{Kir} in cardiac myocytes (Liang *et al.* 2010; Qu *et al.* 2014), and suppresses activity of G protein-coupled inwardly rectifying K^+ channels in dopamine neurons (Sharpe *et al.* 2015); but its effect on K_{ir} channels in HFAs is unknown. Here we evaluated the Meth effect on I_{Kir}. We found that 20 μM Meth did not significantly affect I_{Kir}; but 100 μM Meth significantly increased I_{Kir} (Fig. 6). These results suggest that some K_{ir} channels are functionally expressed in HFAs; but they appeared to be less sensitive to acute effects of Meth at 20 μM level compared to K_{2P} and K_v channels. Chronic Meth effects *in vivo* on dysregulating K_{ir} activity of mature astrocytes need to be investigated in future studies.

Voltage-gated Ca^{2+} currents were not detected in HFAs

We also assessed whether there was a I_{Ca} through VGCCs in HFAs, which were studied under physiological concentration of 2 mM $[Ca^{2+}]_o$. In the Ca^{2+}-related study, voltage-sensitive Na^+, K^+, I_h, and CLC-3 (a voltage-gated chloride channel that conducts outflowing Cl^- currents) were all blocked. We found that when V_m was depolarized from −70 to +50 mV levels, there was no detectable inflowing Ca^{2+} current through VGCCs in HFAs ($p > 0.05$; Figure S1).

Discussion

The present study determined the electrophysiological characteristics of HFAs and acute Meth effects on HFAs. Our findings indicate that (i) HFAs express functional K_{2P}, K_v, and K_{ir} channels, (ii) Meth suppresses I_{Kv}, which is blocked by antagonizing TAAR1, or by inhibiting PKA activity, (iii) Meth at 20 and 100 μM depolarizes RMP of HFAs; and this Meth effect is mimicked and exceeded by blocking K_{2P}/K_{2P}-like channels, (iv) activity of K_{ir}/K_{ir}-like channel is relatively weak and not affected by 20 μM Meth (but reduced by 100 μM of this drug), and (v) there is no functional expression of VGCCs in HFAs. To our knowledge, these novel findings, for the first time in the field, elucidate a mechanism by which acute Meth exposure *in vitro* alters astrocytic K^+ efflux/$[K^+]_o$ by affecting dynamic activity of K^+ channels, which could consequently alter the excitability of surrounding neurons.

The major finding of the present study was that Meth reduced I_{Kv} through K_v/K_v-like channels in HFAs during V_m depolarization. This Meth effect was mimicked by blocking K_v channels with TEA. Importantly, these findings shed light on the underlying mechanism by which Meth suppresses I_{Kv} in HFAs. Specifically, we demonstrate that Meth-induced TAAR1 activation mediates the reduced K_v channel activity, which is reversed by antagonizing TAAR1. It is well established that Meth is a potent TAAR1 agonist (Xie and Miller 2009; Miller 2011; Reese *et al.* 2014; Cotter *et al.* 2015; Liberles 2015). TAAR1 activation promotes the TAAR1/G_s/cAMP/PKA signaling (Jing and Li 2015), which is associated with Meth-induced behavioral alterations (Cotter *et al.* 2015). Phosphorylation of K_v channels by PKA in the cAMP/PKA cascade decreases K_v channel activity in rat cortical neurons (Dong and White 2003; Dong *et al.* 2004), and cardiac myocytes (Walsh and Kass 1988). In agreement with these previous studies, our study reveals that inhibition of PKA activity by antagonizing TAAR1 abolished Meth- induced decrease of I_{Kv} through K_v channels in HFAs. This novel finding indicates that Meth-induced I_{Kv} reduction in HFAs is mediated by the TAAR1-coupled cAMP/PKA signaling. The finding that H89 significantly attenuates Meth-induced I_{Kv} reduction confirms the effect of PKA activation on suppressing K_v/K_v-like channel activity.

Meth-induced reduction in I_{Kv} efflux in HFAs was associated with a reduced time constant (T) during I_{Kv} activation, suggesting that Meth alters the kinetics of K_v channel gating. Interestingly, the T appears to be stabilized in relatively negative V_m levels in HFAs. This may result from the following reasons. First, in *mature* astrocytes, the voltage-dependence of T occurs from RMP (or holding potentials) to V_m levels at which K_v channels begin to activate (~ −60 mV levels). They could achieve their 1/2 activation ($V_{1/2}$) at V_m at ~ −50 mV levels (Zagotta *et al.* 1994; Hille 2001). I_{Kv} depends upon the density, opening probability, and opening time of activated K_A/K_D-like channels, which achieves a steady-status at more depolarized V_m levels (Fig. 2c), at which the T should not be significantly altered by further V_m change. Second, the immaturity of HFAs could affect the property of K_A/K_D channels, including their T (and cause some inconsistency in the V-T relationship). Such immaturity-related variety in RMP mediated by K^+ channels is reported by many previous studies and in the present study. Third, a potential difference in the channel density between K_A channels (which activate/inactivate very fast) and K_D channels (which activate/

inactivate slower than K_A channels; or even don't inactivate), could also affect the *T*. Whether, how, and to what extent these K_A/K_D channels influence the *T* in HFAs (either during activation or inactivation) are not fully understood. Fourth, a lack of the expression of $K_{ir}4.1$ channels in immature astrocytes likely also affects the *T*. The $K_{ir}4.1$ channel is a unique subtype of the inwardly rectifying K^+ channel that is not fully expressed in astrocytes until p15–21 day; but it is the pre-dominant K_{ir} channel subtype in mature astrocytes. Importantly, these unique K_{ir} channels are activated not only during V_m hyperpolarization (like other K_{ir} channels), but also during V_m depolarization (like other K_v channels). Therefore, further investigation is needed to better understand the electrophysiological characteristics of HFAs *ex vivo*, mature astrocytes in the brain, and their responses to the impact of drugs of abuse *in vivo*.

We also found a Meth-induced RMP depolarization, which was mimicked by blocking I_{K2P}. Combined Meth exposure/K_{2P} channel blockade did not induce further RMP depolarization, while inhibiting PKA activity did not affect Meth-induced RMP depolarization. However, there was no significant difference in RMP between control HFAs and those treated by Meth + TAAR1 antagonist, suggesting an involvement of TAAR1. Thus, it is likely that Meth depolarizes RMP by activating TAAR1 and inhibiting K_{2P} channel activity through a mechanism other than the cAMP/PKA cascade in HFAs. Together, these findings suggest that Meth-induced RMP depolarization results from a decreased K_{2P} channel activity and increased intracellular K^+; but such changes are not mediated by the TAAR1-coupled cAMP/PKA signaling. Given that K_{2P} channel activity can be reduced by TAAR1-mediated promotion of PKC activation (Fig. 7), future investigation is also needed to determine if acute Meth effects on depolarizing RMP of HFAs are mediated by the TAAR1-coupled G_q/PLC/PKC signaling pathway.

The present study supports the perspective regarding the *heterogeneity* of astrocyte RMP, which is associated with the immaturity of HFAs. Indeed, the gap junction and some ion channel expression are developmentally regulated (Sontheimer *et al.* 1992; Kressin *et al.* 1995; Giaume and McCarthy 1996; Ben-Ari 2008). This also includes K_{2P}-like channels that play a critical role in regulating RMP *in mature* astrocytes (Olsen et al. 2006; Olsen and Sontheimer 2008). Therefore, RMP of HFAs is much more depolarized in a wider V_m range than those in mature hippocampal, spinal cord, and cortical astrocytes from humans or rats (e.g., RMP = −70 to −80 mV) (Schroder *et al.* 2000; Olsen *et al.* 2006; our unpublished data).

Meth-induced dysfunction alters a variety of K^+ channels, which differs from each other, depending on dosage/blood levels of Meth, cell type, exposure/withdrawal time, and *in vivo/in vitro* exposure (with or without synaptic activity and neurotransmission). These differences can increase or decrease K^+ channels' function. For example, besides suppressing K^+ efflux *via* K_v/K_v-like and K_{2P}/K_{2P}-like channels in HFAs (the present study), Meth also decreases K^+ efflux by inhibiting Ca^{2+}-activated K^+ channel activity in mouse neuroblastoma/rat glioma cells (Wang et al. 2013). Repeated Meth exposure reduces $K_v1.4/1.7/3.4/4.2$ and $K_{ir}2.1/2.2/2.3/2.4$ channels in ventricular myocytes of rats (Qu *et al.* 2014). In contrast, exposure to Meth (≥ 24 h) *increases* K^+ efflux *via* $K_v1.3$ channels in cultured rat microglia (Wang *et al.* 2014); while higher Meth levels (100–1000 μM) cause

dose-dependent microglial cell damage and death). $K_v1.1/K_v1.2$ and their accessory protein KCNAB1 are also upregulated in the NAc of non-addictive rats compared to addictive/compulsive rats following chronic Meth-SA (Cadet *et al.* 2016). Therefore, further studies are needed to confirm or modify the 'recreational' and 'toxic/lethal levels of Meth in the blood of drug users. It will also be interesting to assess the blood levels of Meth in rats that self-administer the drug.

The importance of Meth-induced alterations in astrocytic K^+ channels results from its influence on the extracellular K^+ homeostasis, and consequently on neuronal activity. Meth-induced decrease in astrocytic K_{2P}/K_v channel function will reduce I_K outflowing from HFAs; and therefore lower $[K^+]_o$. Based on the Goldmann-Hodgkin Katz voltage equation (Hille 2001), decreased K^+ efflux/$[K^+]_o$ facilitates V_m hyperpolarization of surrounding neurons, and consequently reduce their firing (Fig. 7). Thus, it is very likely that such an altered astrocyte/neuron interaction in the brain will contribute to the underlying mechanism of Meth neuropatho-physiology. In agreement with this hypothesis, our previous studies demonstrate a significant decrease of neuronal activity in the rat medial prefrontal cortex and NAc following chronic Meth-SA (Chen *et al.* 2012; Graves *et al.* 2015), or repeated Meth subcutaneous injections (White *et al.* 1995; Hu *et al.* 2002), respectively.

The present study also revealed the functional expression of K_{ir} channels in HFAs. Compared to I_{Kv}, astrocyte I_{Kir} was relatively small, which is in agreement with other studies of *immature* astrocytes (Kressin *et al.* 1995; Olsen *et al.* 2006; Olsen and Sontheimer 2008; Montiel-Herrera and Garcia-Colunga 2010). Intriguingly, unlike RMP and I_{Kv}, I_{Kir} in HFAs was not affected by 20 μM Meth; but reduced by toxic/fatal concentrations of Meth (100 μM). This finding suggests that K_{ir}/K_{ir}-like channels are less sensitive, but still vulnerable, to the acute Meth compared to K_{2P}/K_{2P}-like and K_v/K_v- like channels in HFAs. However, we also acknowledge that the Meth effects (including its selectivity) on astrocytes (as well as their K^+ channel activity), either *in vitro* or *in vivo*, are not fully understood. The present study alone will not be able to address/answer all the questions regarding the impact of high concentrations of Meth on all subtypes of K^+ channels. Given that this study is the first one of its kind in the field (up to the time of submission), we hope that our novel findings will inspire more investigations to define the mechanism(s) by which drugs of abuse alter the functional activity of astrocytes and neurons *in vivo*, in laboratory animals and humans. Intriguingly, in our previous studies (Nasif *et al.* 2005b; Wayman *et al.* 2015), we have also demonstrated a lack of selectivity regarding cocaine-induced alterations in various K^+ channel subtypes in rat cortical neurons.

In summary, the present study demonstrates that the HFA activity is dynamically regulated by a variety of K^+ channel subtypes at different V_m levels. Meth at a recreational level reduces astrocytic K^+ efflux through K_{2P}/K_{2P}-like and K_v/K_v-like channels. Such K_v/K_v-like channel dysfunction is mediated by the TAAR1-coulped cAMP/PKA cascade; while K_{2P}/K_{2P}-like channel dysfunction may also be mediated by other mechanisms. Together, these Meth-induced alterations in K^+ channels disturb astrocyte regulation of $[K^+]_o$, which could consequently decrease excitability of surrounding neurons in the brain regions that are vulnerable to Meth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments and conflict of interest disclosure

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Abbreviations used:

$[K^+]_o$	extracellular K^+ level
CABM	clonetics astrocyte basal medium
cAMP	cyclic adenosine monophosphate
C_m	membrane capacitance
CNS	central nervous system
EPPTB	*N*-(3-Ethoxyphenyl)-4-(1-pyrrolidinyl)-3-(trifluoromethyl)benzamide
G_q	G_q protein
G_s	G_s protein
HFA	human fetal astrocyte
I_h	the hyperpolarization-activated channel current
I_{K2P}	the ‘leaking’ K^+ current
I_{Kir}	the inwardly rectifying K^+ current
I_{kv}	the voltage-sensitive K^+ current
K_{2P}	channel, the two-pore domain K^+ channel
K_{ir}	channel, the inwardly rectifying K^+ channel
K_v channel	the voltage-gated K^+ channel
LUHMES	Lund human mesencephalic neurons
Meth	methamphetamine
Meth-SA	Meth self-administration
mPFC	medial prefrontal cortex
ms	millisecond
NAc	nucleus accumbens

PKA	cAMP-activated protein kinase
PKC	protein kinase C
PLC	phospholipase C
QUIN	quinidine
R_{in}	input resistance
RMP	resting membrane potential
RM	repeated measurement
RRID	research resource identifier
SA	self-administration
TAAR1	the trace amine-associated receptor type-1
TEA	tetraethyl-ammonium
TTX	tetrodotoxin
VGCC	voltage-gated Ca^{2+} current
V_h	holding potential
V_m	membrane potential

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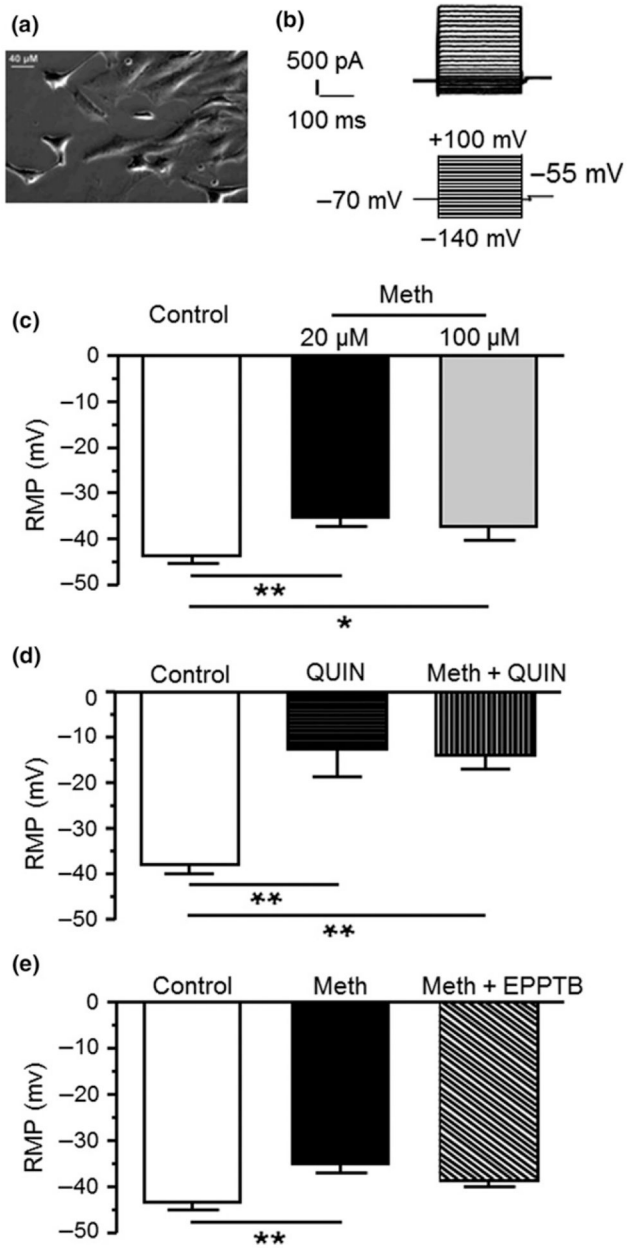


Fig. 1.

(a) Cultured human fetal astrocytes (HFAs) in a phase-contrast image. (b) The top panel shows representative current traces from an untreated HFA in response to membrane hyperpolarization (downward) or depolarization (upward). The lower panel shows the recording protocol (V_m clamping) used for assessing I_K in HFAs. (c) Resting membrane potential (RMP) was depolarized in response to 20 and 100 µM methamphetamine (Meth) compared to vehicle-treated control (Control vs. 20 and 100 µM Meth, n = 37 vs. 17 and 12 HFAs (cells) from 29, 14, and 8 independent experiments, respectively; One-way ANOVA: $F_{(2,63)}$ = 6.119, p = 0.004; with Newman–Keuls *post hoc* test: *,**p < 0.05 or 0.01). (d) K_{2P} channel blockade (QUIN, 1 mM) also induced RMP depolarization in control cells. There was no further RMP depolarization in HFAs treated with Meth + quinidine (n = 4/4 cells/

independent experiments for each group; One-way ANOVA: $F_{(2,9)} = 13.1$, $p = 0.002$; with Newman–Keuls *post hoc* test: $^{**}p < 0.01$). (e) Meth-induced RMP depolarization was partially attenuated by combined treatment with TAAR1 antagonist *N*-(3-Ethoxyphenyl)-4-(1-pyrrolidinyl)-3-(trifluoromethyl)benzamide (EPPTB) (25 nM) (control vs. Meth, and Meth + EPPTB, $n = 37/29$ vs. 17/14 and 9/4 cells/independent experiments, respectively; One-way ANOVA: $F_{(2,60)} = 6.598$, $p = 0.003$; with New-man Keuls *post hoc* test, $^{**}p < 0.01$). There was no significant difference in RMP between control cells and those treated with Meth plus TAAR1 antagonist ($p = 0.181$).

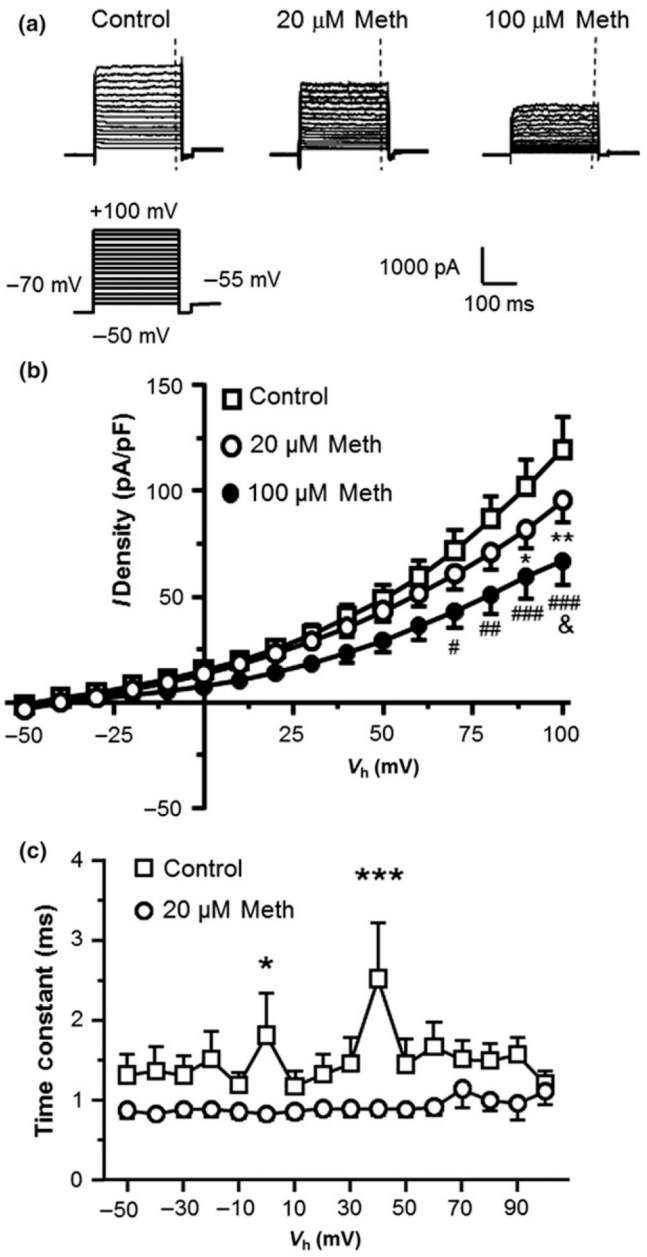


Fig. 2.

Voltage-sensitive outflowing I_{Kv} during V_m depolarization were significantly reduced in response to acute methamphetamine (Meth) exposure. (a) Sample traces of I_{Kv} at control condition, and in response to 20, and 100 μM Meth. The vertical dashed lines indicate the time points at which the currents were measured. (b) Acute Meth exposure *in vitro* induced a significant decrease in the voltage-sensitive outflowing I_{Kv} (Control vs. 20 and 100 μM Meth, n = 22/18 vs. 19/12 and 9/7 cells/independent experiments, respectively; Two-way rmANOVA: treatment: $F_{(2,47)} = 2.775$, $p = 0.0726$; voltage: $F_{(15,705)} = 106.7$, $p < 0.001$; interaction: $F_{(30,705)} = 2.784$, $p < 0.001$. Tukey's post hoctest: $^{*,**}p < 0.05$ or 0.01 for Control vs. Meth 20 μM, $^{\#,\#\#,\#\#\#}p < 0.05$, 0.01, or 0.001 for Control vs. 100 μM Meth, and $^{\&}p < 0.05$ for 20 μM Meth vs. 100 μM Meth). (c) Acute Meth significantly reduced the time

constant (T) of I_{Kv} during the activation of K_v/K_v-like K^+ channels in human fetal astrocytes compared to controls (Control: $n = 20/18$ vs. Meth: $n = 14/10$ cells/independent experiments; Two-way rmANOVA: treatment: $F_{(1,32)} = 5.377$, $p = 0.027$; voltage: $F_{(15,467)} = 1.028$, $p > 0.05$; interaction: $F_{(15,467)} = 1.143$, $p > 0.05$. Newman–Keuls *post hoc* test: *,***$p < 0.05$ or 0.001).

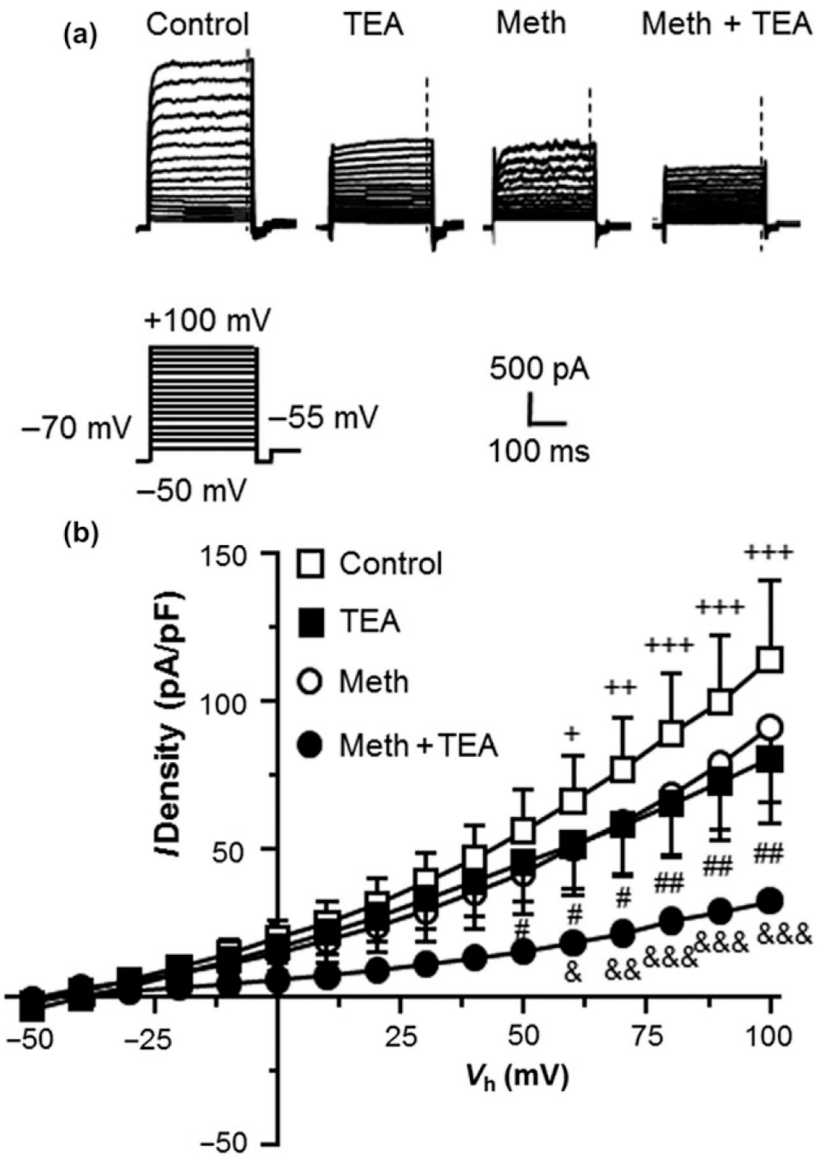


Fig. 3.

I_{Kv} efflux through K_v channels was significantly reduced by methamphetamine (Meth) and tetraethyl-ammonium (TEA). (a) Sample traces of I_{Kv} in human fetal astrocytes, with or without Meth (20 μM) and/or TEA. The vertical dashed lines indicate the time points at which the currents were measured. (b) The *I-V* curves show that both TEA and Meth decreased I_{Kv} by blocking K_v channels; and that a significantly greater reduction was induced by combined exposure to TEA and Meth (Control and TEA, $n = 6/5$ cells/independent experiments; Meth and Meth + TEA, $n = 4/3$ cells/independent experiments; Two-way rmANOVA; Control vs. TEA: treatment: $F_{(1,5)} = 11.01$, $p = 0.021$; voltage: $F_{(15,75)} = 16.81$, $p < 0.001$; interaction: $F_{(15,75)} = 7.088$, $p < 0.001$; with Newman–Keuls *post hoc* test: $^{+,++,+++}p < 0.05$, 0.01 or 0.001; Meth vs. Meth + TEA: treatment: $F_{(1,3)} = 3.519$, $p = 0.157$; voltage: $F_{(15,45)} = 27.83$, $p < 0.001$; interaction: $F_{(15,45)} = 4.308$, $p < 0.001$; with Newman–Keuls *post hoc* test: $^{\&,\&\&,\&\&\&}p < 0.05$, 0.01 or 0.001; TEA vs. Meth + TEA: treatment:

$F_{(1,8)} = 2.947$, $p = 0.124$; voltage: $F_{(15,120)} = 15.47$, $p < 0.001$; interaction: $F_{(15,120)} = 3.027$, $p < 0.001$; with Newman–Keuls *post hoc* test: $^{\#,\#\#}p < 0.05$ or 0.01).

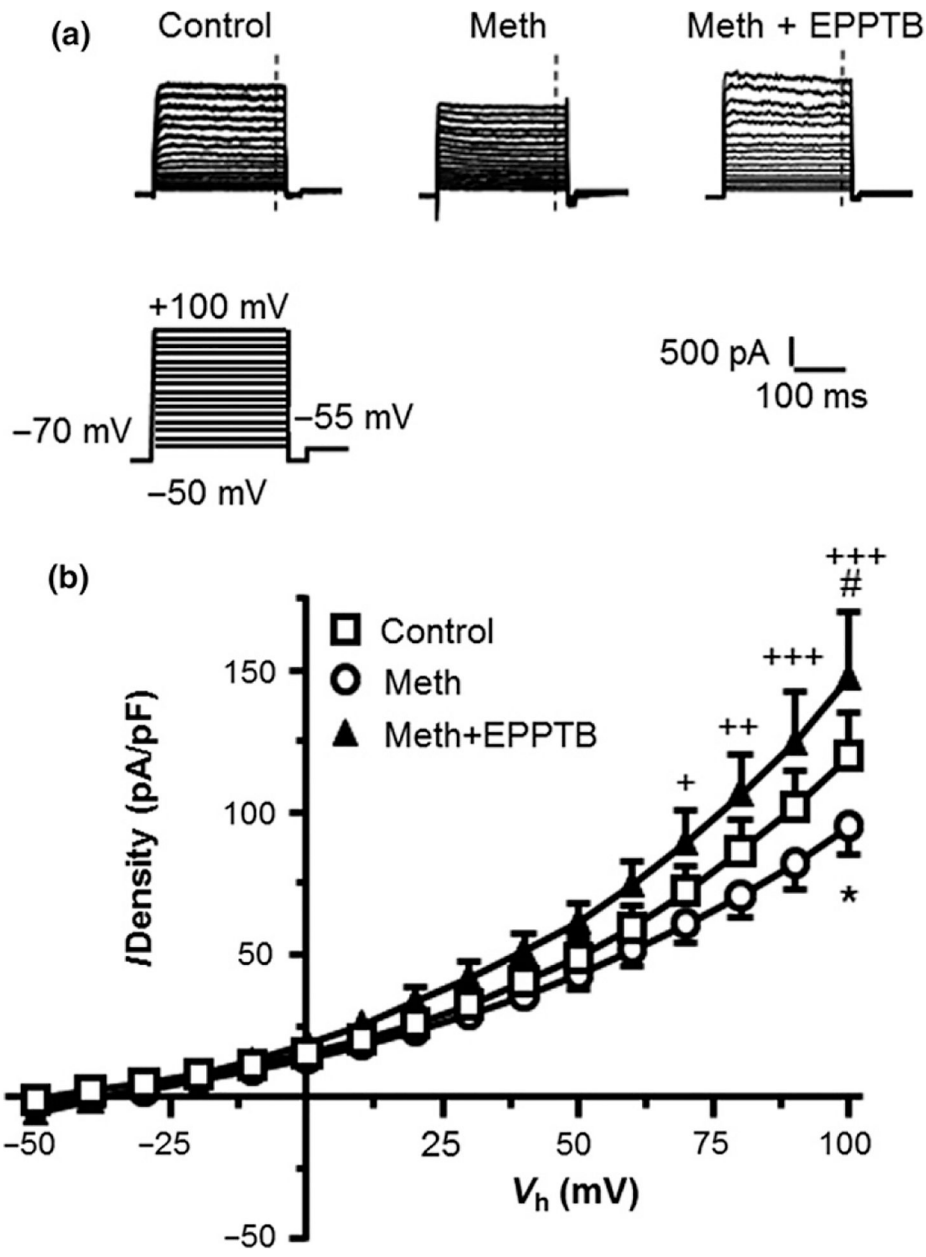


Fig. 4.

Antagonism of TAAR1 by *N*-(3-Ethoxyphenyl)-4-(1-pyrrolidinyl)-3-(trifluoromethyl)benzamide (EPPTB) reversed acute effects of methamphetamine (Meth) on suppressing I_{Kv}. (a) Sample traces from human fetal astrocytes (from the left to right) at control condition, treated with Meth (20 μM), or with Meth (20 μM) plus EPPTB (25 nM). The vertical dashed lines indicate the time points at which the currents were measured. (b) The *I-V* curves show that combined treatment of EPPTB and Meth reversed the Meth-induced decrease in I_{Kv} (Control vs. Meth and Meth + EPPTB: n = 22/18 vs. 19/12 and 9/4 cells/independent experiments, respectively; Two-way rmANOVA: treatment: $F_{(2,47)} = 1.786$, $p = 1.786$; voltage: $F_{(15,705)} = 155.7$, $p < 0.001$; interaction: $F_{(30,705)} = 2.112$, $p < 0.001$; with

Tukey's *post hoc* test: Control vs. Meth, $*p < 0.05$; Control vs. Meth + EPPTB, $^{\#}p < 0.05$; Meth vs. Meth + EPPTB: $^{+,++,+++}p < 0.05$, 0.01 or 0.001).

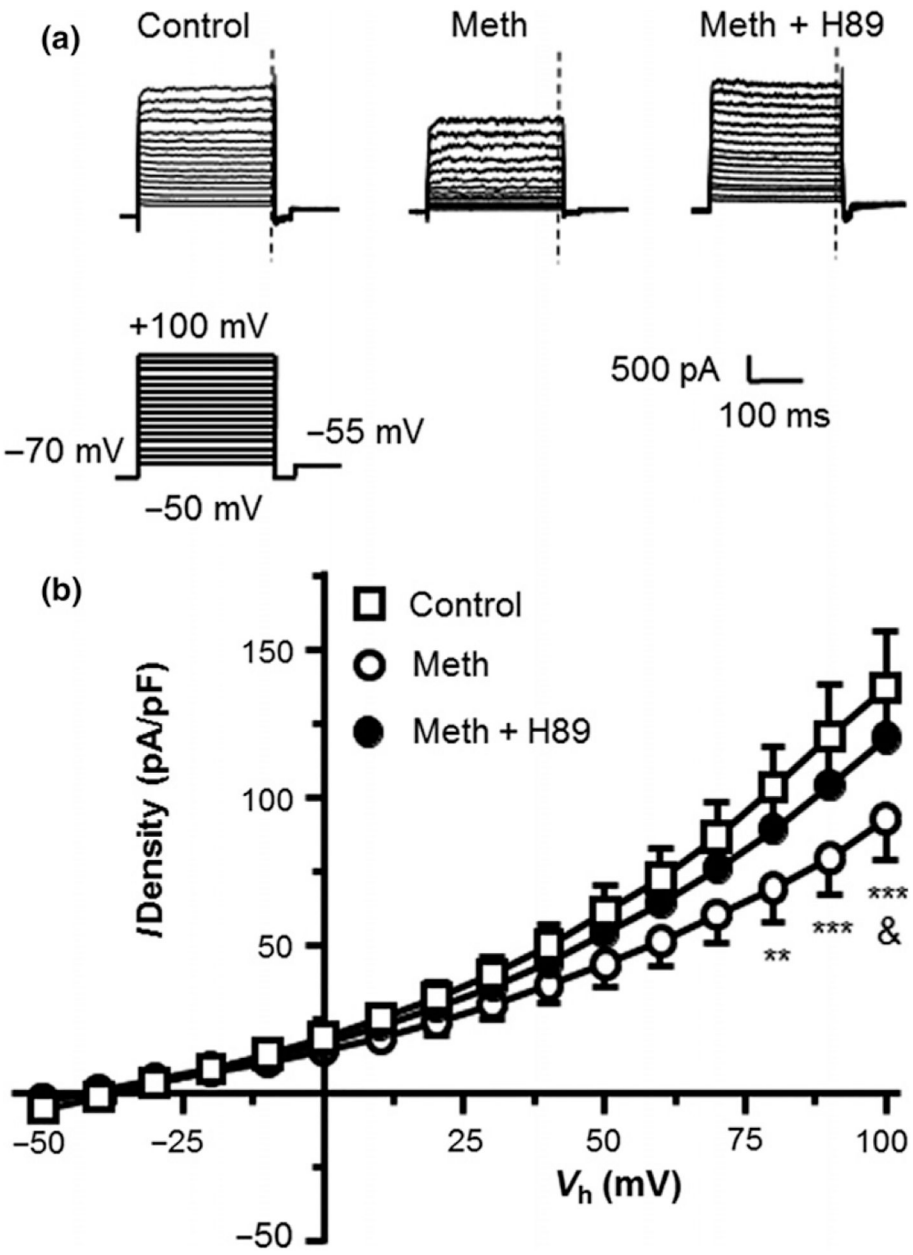


Fig. 5.

PKA inhibition by H89 abolished acute effects of methamphetamine (Meth) on I_{Kv}. (a) Sample traces from human fetal astrocytes at control condition, treated with Meth (20 μM), or with Meth (20 μM) plus H89 (10 μM). The vertical dashed lines indicate the time points at which the currents were measured. (b) The *I-V* curves show that combined treatment of H89 and Meth reversed the Meth-induced reduction in I_{Kv} (Control vs. Meth & Meth + H89: n = 5/4, 5/5, 5/5 cells/independent experiments, respectively; Two-way rmANOVA: treatment: $F_{(2,12)} = 1.343$, $p > 0.05$; voltage: $F_{(15,180)} = 157.6$, $p < 0.001$; interaction: $F_{(30,180)} = 2.178$, $p < 0.001$; with Tukey's *post hoc* test: Control vs. Meth: **,***$p < 0.01$, or 0.001; Meth vs. Meth + H89: $^{\&}p < 0.05$).

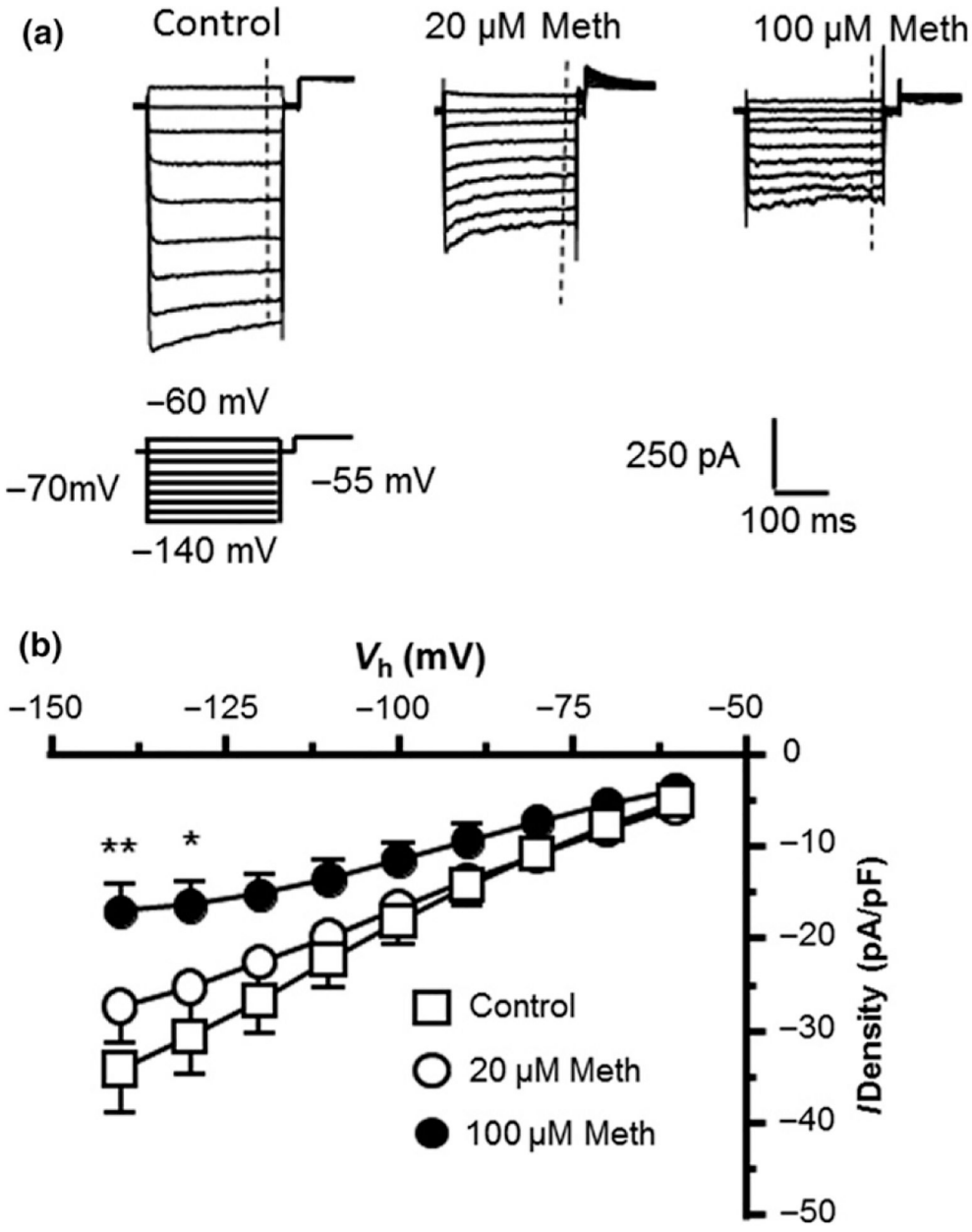
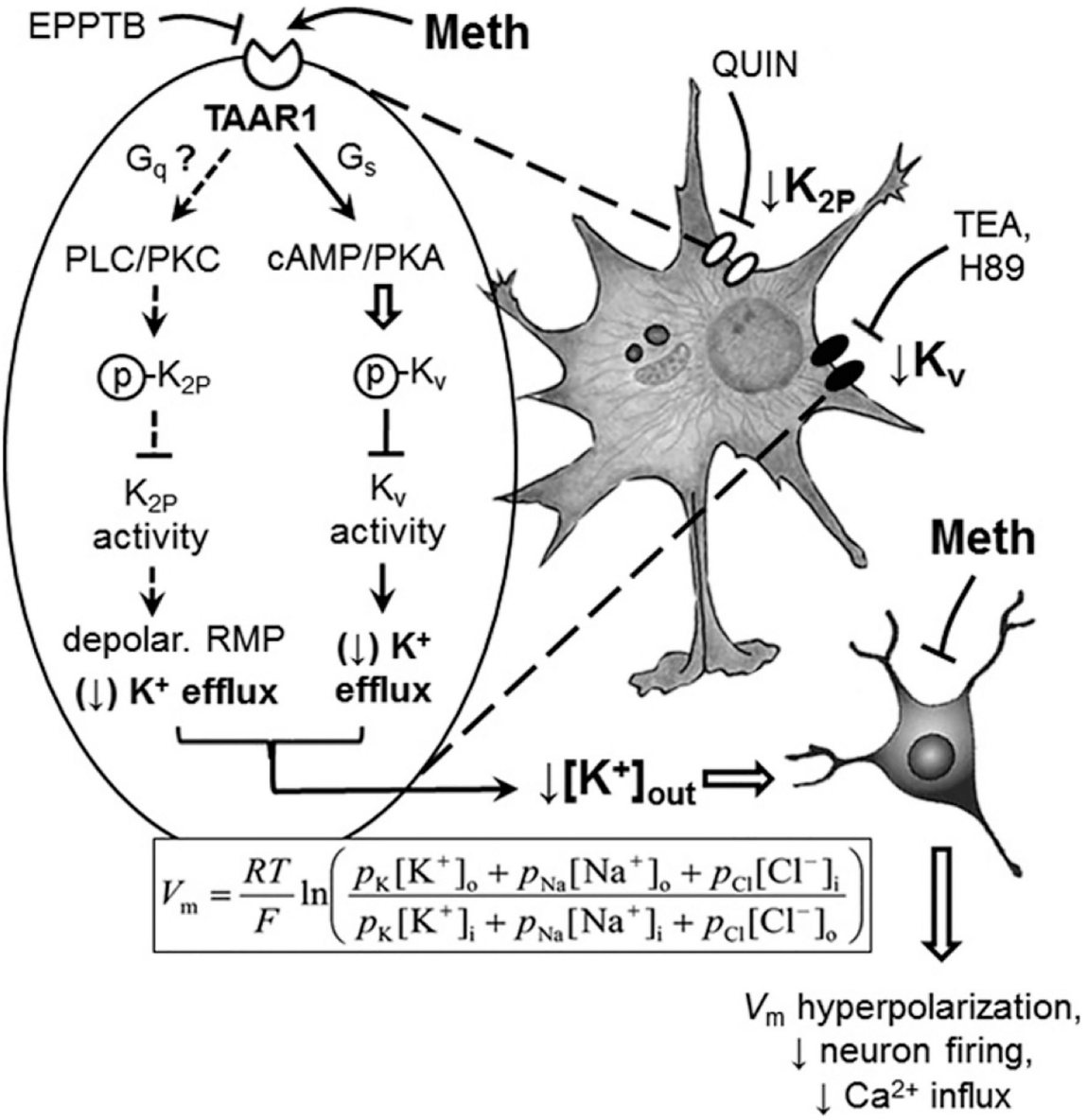


Fig. 6.

Methamphetamine (Meth) at higher (but not lower) concentration increased inwardly rectifying K^+ currents in human fetal astrocytes (HFAs). (a) Sample traces of inwardly rectifying K^+ currents (I_{Kir}) indicate that 100 μM (but not 20 μM) Meth increased I_{Kir} in HFAs. (b) The *I-V* relationships reveal that I_{Kir} was significantly increased by 100 μM Meth compared to controls (Control vs. 20 and 100 μM Meth, n = 32/26 vs. 17/12 and 9/7 cells/independent experiments; Two-Way rmANOVA: Treatment effect: $F_{(2,55)} = 1.41$, $p > 0.05$; Voltage effect: $F_{(8,440)} = 60.91$, $p < 0.001$; interaction: $F_{(16,440)} = 2.889$, $p = 0.0002$; with Newman–Keuls *post hoc* test: *,**p < 0.05 or 0.01).



$$V_m = \frac{RT}{F} \ln\left(\frac{p_K[K^+]_o + p_{Na}[Na^+]_o + p_{Cl}[Cl^-]_i}{p_K[K^+]_i + p_{Na}[Na^+]_i + p_{Cl}[Cl^-]_o}\right)$$

Fig. 7.

Acute methamphetamine (Meth) effects on altering astrocyte activity are mediated by TAAR1-coupled signaling and K^+ channel dysfunction. Astrocytes play a key role in regulating extracellular K^+ homeostasis in the brain. Meth alters this dynamic function of astrocytes in regulating K^+ homeostasis by suppressing K^+ efflux through dysfunctional K_v, K_{2P} and K_{ir} channels. Acute Meth effects on altering K^+ efflux through K_v channels are mediated by disrupting the TAAR1-coupled cAMP/PKA signaling pathway. The mechanism underlying acute Meth effects on suppressing K_{2P} channel activity [leading to resting membrane potential (RMP) depolarization] could be mediated by dysfunction of the G_s/cAMP/PKA and/or G_q/phospholipase C (PLC)/protein kinase C (PKC) signaling pathway. Meth is a potent agonist for TAAR1. Direct activation of TAAR1 by Meth promotes the TAAR1/G_s/cAMP/PKA signaling pathway, as well as the TAAR1/G_q/PLC/PKC signaling

pathway, thereby enhancing PKA/PKC-induced phosphorylation of K_v/K_{2P} channels, respectively. Enhanced phosphorylation of K_v/K_{2P} channels reduces their activity to conduct outflowing K^+. Reducing K^+ efflux from astrocytes via K_v/K_{2P} channels decreases extracellular K^+ levels ($[K^+]_o$). The Goldman-Hodgkin Katz equation indicates that reduced $[K^+]_o$ results in V_m hyperpolarization of surrounding neurons; and therefore decreases their excitability. The mechanism, by which toxic/fatal level of Meth suppresses K_{ir} channel activity, remains to be identified. These findings suggest that Meth disturbs functional and dynamic activity of astrocytes by altering the functional activity of K_v and K_{2P} channels through the cAMP/PKA and likely PLC/PKC signaling pathway, respectively; and that could affect their interactions with neurons in the brain.