Crosstalk between AQP4-dependent ATP/Adenosine release and dopamine neurotransmission in depressive behavior during cocaine withdrawal

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Abstract

The brain abundantly expresses adenosine receptors, which are involved in the regulation of neural activity, blood flow, and inflammation. In a previous study using our originally developed adenosine biosensor, we reported that hippocampal astrocytes release ATP upon water influx from the water channel AQP4, which is degraded extracellularly to increase adenosine (Yamashiro et al., 2017). On the other hand, the interaction between adenosine and dopamine is widely known, and when adenosine release from astrocytes is altered by inflammation or other factors, abnormal dopamine neurotransmission and related ataxia and psychiatric disorders may develop. In the present study, we examined pathological changes in adenosine or dopamine release in depressive-like behavior that develops as a symptom of cocaine withdrawal. The results showed that A_1 receptor inhibitors and AQP4 gene disruption suppressed depressive-like behavior. In the striatum, AQP4-dependent adenosine release inhibited dopamine release via A_1 receptors, and cocaine inhibited dopamine release by increasing this adenosine release. In contrast, in the medial frontal cortex, AQP4-dependently released adenosine enhanced dopamine release via A_1 receptors, and cocaine abolished this adenosine effect. Furthermore, adenosine action was restored in AQP4 knockout mice, suggesting that cocaine reduced A_1 receptor function via AQP4-dependent adenosine. In conclusion, astrocytes modulate dopaminergic neurotransmission through AQP4-mediated adenosine release, and this disruption leads to depression-like behavior.

Introduction

Adenosine, a nucleic acid metabolite, modulates neural activity through receptor-mediated actions. Adenosine action is mediated by G protein-coupled receptors, A_1, $_{A2A}$, A_{2B}, and A_3, with A_1 and A_{2A} receptors being expressed mainly in the brain (Chen et al., 2001). A_1 receptors are widely expressed in the brain and are involved in presynaptic inhibition of glutamate and dopamine (Chen et al., 2013; Yabuuchi et al., 2006). In contrast, A_{2A} receptors are G_s-coupled and are abundantly expressed in the basal ganglia, including the striatum, where they influence dopamine neurotransmission (Ferré et al., 1997) and are involved in the maintenance of synaptic plasticity as a cofactor for BDNF (Fontinha et al., 2008). In addition to this, adenosine has sleep-promoting, vasorelaxant, and anti-inflammatory effects in the brain (Haskó et al., 2008; Lazarus et al., 2011; Ralevic and Dunn, 2015).

Much remains unknown regarding the regulation of extracellular adenosine levels in the brain. Using strain cells that exhibit intracellular calcium elevation in response to adenosine as biosensors, our group found that adenosine release in hippocampal slices is mainly derived from two pathways (Yamashiro et al., 2017). Namely, the pathway in which dendrites of pyramidal neurons release adenosine in response to electrical activity and the pathway in which ATP is released in response to hypoosmotic treatment in a manner dependent on the astrocyte-specific expressed water channel AQP4, which is degraded by extracellular enzymes to increase adenosine. Because astrocytes express AQP4 abundantly (Nielsen et al., 1997), osmotic changes are thought to efficiently induce volume changes. In addition, almost all cells, including astrocytes, have high plasma membrane permeability for K^+, so if anion channels are activated upon expansion, intracellular ions are quickly released and can adapt to osmotic changes. Such an osmotic adaptation mechanism is called regulatory volume decrease (RVD), and the anion channels that are activated upon expansion are

known as volume-regulated anion channels (VRAC) (Okada et al., 2001). The AQP4-dependent ATP release upon hypoosmotic treatment reflects the RVD of astrocytes, and ATP is thought to be released via VRAC. However, since the gene for VRAC has not yet been identified and selective inhibitors have not yet been established, studies of VRAC have been limited to its electrophysiological properties.

The biosensor used in the previous study was a HEK293 cell expressing A_1 receptor and Gqi5 (a fusion protein of G_i and G_q, which co-activates G_i-coupled receptors to elevate intracellular calcium)), which reproducibly shows $[Ca^{2+}]_i$ elevation in response to adenosine but has problems with quantitation due to pronounced desensitization. To improve this point, a new biosensor, A_1-CHO, was created by expressing the A_1 receptor, Gqi5, and the calcium fluorescent protein RCaMP (Inoue et al., 2014) in CHO cells (Palmer et al., 1996), in which phosphorylation of the A_1 receptor does not occur and confirmed that there was no desensitization (Masato Kobayashi, Master's thesis 2019). We also used the same method to create D_2-CHO, a biosensor for dopamine. By using them, we achieved quantification of adenosine and dopamine with high quantitatively and without the need for calcium fluorochrome staining.

The interaction between adenosine and dopamine is widely known. Dopamine is a neurotransmitter that projects mainly from the ventral tegmental area and substantia nigra of the midbrain to the cerebral cortex, limbic system, and basal ganglia, and is involved in motivation, emotion, learning, motor regulation, and hormone regulation A_{2A} receptors, like D_1 and D_2 receptors, are abundantly expressed in basal ganglia, including striatum (Chen et al, 2001), and A_{2A} and D_2 receptors form an antagonistic heterodimer (Hillion et al., 2002). This has already been exploited in the treatment of Parkinson's disease, an ataxia associated with decreased dopamine neurotransmission in the striatum,

using blocking agents of A_{2A} receptors to promote dopamine neurotransmission (Kondo and Mizuno, 2015). In contrast, A_1 receptors inhibit dopamine release in the striatum and spinal cord (Acton et al., 2018; Ross and Venton, 2015).

Modulation of dopamine neurotransmission is a known cause of psychiatric disorders such as bipolar disorder and schizophrenia as well as Parkinson's disease. In particular, a number of studies have recently reported that neuroinflammation caused by stress and drug dependence decreases dopamine neurotransmission in the striatum, including the medial frontal cortex and the nucleus accumbens, causing depressive symptoms (Brites and Fernandes, 2015; Cotto et al., 2018; Furuyashiki, 2012). On the other hand, astrocytes show diverse activation upon inflammation, and it is quite possible that adenosine release is altered in astrocytes activated by neuroinflammation. An example of increased expression of adenosine degrading enzymes in activated astrocytes leading to decreased adenosine and consequently to epilepsy due to increased glutamate release is already known (Fedele et al., 2005). In contrast, astrocyte atrophy has been reported in depressed patients (Zhao et al., 2018), and it is quite possible that an increase in adenosine, the opposite of epilepsy, occurs. Based on the above, the present study hypothesizes that an AQP4-dependent increase in astrocyte adenosine release suppresses dopaminergic neurotransmission and leads to the development of depression. To demonstrate this hypothesis, we used depression-like behavior in mice, which develops as a symptom of cocaine withdrawal (Filip et al., 2006), as a model and investigated adenosine and dopamine release using biosensors, focusing on the medial frontal cortex and striatum, the major dopamine nerve projection sites in the brain. In addition, AQP4 knockout mice were used to examine AQP4-dependent adenosine release as distinct from other release pathways. This study is expected to lead to new research for the diagnosis and treatment of depression if the actual conditions behind

the changes in adenosine release and astrocyte activation that underlie depressive-like behavior are elucidated.

Materials & Methods

Animal experiments

All animal experiments were approved by the institutional animal care and use committee of Kobe University (Permission number: 25-09-04) and performed in compliance with the Kobe University Animal Experimentation Regulations. C57BLC background mice were maintained at 24℃ and under 12h light/dark cycle. Water and food were provided ad libitum. Mice at least 8 weeks old were used for experiment. AQP4 knock out mice ($AQP4^{-/-}$) (Kitaura et al., 2009) were provided by Dr. Mika Terumitsu (National Center of Neurology and Psychiatry, Tokyo, Japan). Depressive behavior were examined between 14 days to 17 days after five consecutive days intraperitoneal injection of 15 mg/kg cocaine. For forced swim test, mice were acclimatized in an experiment room with white noise for one hour, then placed in water (24℃), which was 12.5 cm high in a transparent cylinder of 22 cm in diameter (Can et al., 2012). 8 min RGB videos at 15 fps were taken by an web camera, C270n (Logicool, Tokyo, Japan). Mobility of mice were quantified as described by Gao et al (Gao et al. 2014). Images in red color channel were converted to 8 bit gray-scale and each frame were subtracted by previous ones. Then pixels above a threshold of five digit were counted in each frame and averaged for 15 consecutive frames. Since this value reflect the mobility of mice in one second, immobility time were measured as the duration, in which the value was below a threshold determined by an experimenter for each test. The immobility time was plotted every 60 sec.

Biosensor

CHO cells were maintained in Ham's F12 medium containing 10% fetal bovine serum and antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin). For $[Ca^{2+}]_i$ imaging, cells were seeded on coverslips. As biosensors for adenosine or dopamine, stable CHO cell lines expressing RCaMP, $G_{qi}5$ and mouse A_1 receptor or human D2 receptor were established by transfecting CHO cells with pCAG-cyto-RCaMP (Addgene), pME- $G_{qi}5$ (Yamashiro et al***) and pCMV-mA1 (OriGene) or DRD2-Tango (Addgene), screening with 200 ug/mL G418 and $[Ca^{2+}]_i$ imaging to select clonal cells showing $[Ca^{2+}]_i$ increase to 100 μM adenosine or 100 μM dopamine.

Brain slice preparation

Mice were anesthetized mice by intramuscular injection of ketamine (100mg/kg, Daiichi-sankyo, Tokyo, Japan) and xylazine (10 mg/kg, Bayer Yakuhin, Osaka, Japan) and decapitated. Brains were extracted and put into ice-cold slice cutting (2 mM KCl, 1.5 mM NaH_2PO_4, 26 mM $NaHCO_3$, 6 mM $MgSO_4$, 200 μM $CaCl_2$ 10 mM D-glucose, 220 mM sucrose) saturated with 95% O_2 and 5% CO_2. Coronal slices of 400μm thickness were obtained by cutting with ZERO-1 microslicer (Dosaka EM, Kyoto, Japan) and maintained in artificial cerebrospinal fluid (aCSF; 124 mM NaCl, 2 mM KCl, 1.5 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1 mM $MgSO_4$, 10 mM D-グルコース, 2 mM $CaCl_2$) for more than one hour before experiment.

Calcium imaging

Slices were perfused with aCSF at 5 mL/min. RCaMP images of biosensor were obtained every one second by using an inverted microscope (IX-70, Olympus, Tokyo, Japan) equipped with an objective (UApo/340 20x / 0.70, Olympus) and a cooled-CCD camera (Orca-R2, Hamamatsu Photonics, Hamamtsu, Japan), and F/F_0 was calculated by ImageJ. Electrical stimulation was given by house-

made concentric electrode, isolator (ISO-Flex, Funakoshi, Tokyo, Japan) and pulse generator (Electronic Stimulator, Nihon Koden, Tokyo, Japan). For hypo-osmotic treatment, the aCSF of the osmolarity indicated were prepared by reducing NaCl concentration. Slices were pretreated with the low NaCl aCSF, which was supplemented with sucrose to achieve iso-osmolarity.

Reagents

If not specified, reagents purchased from nacalai tesque (Kyoto, Japan).

Statistical analysis

For statistical analysis by Excel 2016, 50 cells were randomly selected from each slice in Fig2BC and 5AC while eight responding cells were randomly selected in Fig3, 4, 5BD, 6, 7 and 8.

Results

The involvement of AQP4-dependent adenosine increases in depressive behavior during cocaine withdrawal.

The involvement of AQP4-dependent adenosine in depressive behavior was examined. Depressive behavior was induced by cocaine withdrawal and measured as immobility time during forced swim test. Immobility times of wild type mice treated with saline (WT / (-)) or cocaine (WT / Cocaine), were 21.33±13.00 sec (WT / (-)) or 35.82±12.08 sec (WT / Cocaine) between 2 and 3 min after starting test, 19.53±14.09 sec (WT / (-)) or 36.74±8.59 sec (WT / Cocaine) between 3 and 4 min, which indicated significant increase by cocaine (Fig. 1A).

This depressive behavior was abolished in $AQP4^{-/-}$ (Fig. 1B). Instead, immobility times of saline ($AQP4^{-/-}$ / (-)) or cocaine ($AQP4^{-/-}$ / Cocaine) treated groups were 34.77±10.67 sec ($AQP4^{-/-}$ / (-)) or

14.83±16.25 sec (AQP4$^{-/-}$ / Cocaine) between 4 and 5 min after stating test, 36.07±10.53 sec (AQP4$^{-/-}$ / (-)) or 20.92±15.94 sec (AQP4$^{-/-}$ / Cocaine) between 5 and 6 min, 38.28±12.06 sec (AQP4$^{-/-}$ / (-)) or 20.57±14.10 sec (AQP4$^{-/-}$ / Cocaine) between 6 and 7 min, which showed significant reduction by cocaine (Fig. 1B). Furthermore, the involvement of adenosine was examined by a non-selective adenosine receptor antagonist caffeine. Saline or 50 mg/kg caffeine was administered to cocaine treated mice 40 min prior to forced swim test. Immobility times of wild type mice treated with cocaine (WT / Cocaine / (-)) or cocaine and caffeine (WT / Cocaine / Caffeine) treated groups were 15.64 ± 7.78 sec (WT / Cocaine / (-)) or 5.74 ± 7.19 sec (WT / Cocaine / (-) / Caffeine) between 1 and 2 min after starting test, 27.61 ± 6.42 sec (WT / Cocaine / (-)) or 9.70 ± 7.31 sec (WT / Cocaine / (-) / Caffeine) between 2 and 3 min, 23.42±5.39 sec (WT / Cocaine / (-)) or 6.33 ± 8.71 sec(WT / Cocaine / (-) / Caffeine) between 3 and 4 min, 31.56 ± 9.35 sec (WT / Cocaine / (-)) or 9.85 ± 11.43 sec (WT / Cocaine / (-) / Caffeine) between 4 and 5 min, 39.56 ± 8.47 sec (WT / Cocaine / (-)) or 13.50±10.11 sec (WT / Cocaine / (-) / Caffeine) between 5 and 6 min, 39.82 ± 10.29 sec (WT / Cocaine / (-)) or 21.58 ± 13.59 sec (WT / Cocaine / (-) / Caffeine) between 6 and 7 min, 44.94 ± 3.37 sec (WT / Cocaine / (-)) or 19.66 ± 13.22 sec (WT / Cocaine / (-) / Caffeine) between 7 and 8 min were significantly reduced in caffeine and nicotine treated mice (Fig. 1C). The effect of caffeine was likely mediated by adenosine A_1 receptor, because an A_1-selective antagonist, DPCPX also, which were treated at 4mg/kg, 30 min prior to forced swim test, abolished depressive behavior. Immobility times of wild type mice treated with cocaine (WT / Cocaine / (-)) or cocaine and DPCPX (WT / Cocaine / DPCPX) were 6.48 ± 5.94 sec (WT / Cocaine / (-)) or 0 ± 0 sec (WT / Cocaine / DPCPX) between 1 and 2 min, 21.65 ± 11.55 sec (WT / Cocaine / (-)) or 6.17 ± 7.01 sec (WT / Cocaine / DPCPX) between 3 and 4 min, 37.71 ± 5.49 sec (WT / Cocaine / (-)) or 5.67 ± 6.39 sec (WT / Cocaine / DPCPX) between 5 and 6 min,

36.75 ± 14.42 sec (WT / Cocaine / (-)) or 5.68 ± 7.04 sec (WT / Cocaine / DPCPX) between 6 and 7 min, 40.28 ± 14.14 sec (WT / Cocaine / (-)) or 20.97 ± 10.60 sec (WT / Cocaine / DPCPX) between 7 and 8 min were significantly reduced in DPCPX and cocaine treated mice (Fig. 1D) suggesting involvement of A_1 receptor activation in depressive behavior. Overall, these results suggest the involvement of AQP4 and adenosine A_1 receptor in depressive behavior during cocaine withdrawal.

Adenosine releases in the striatum

Ventral part of striatum including nucleus accumbens were subjected to the analysis of adenosine release by A_1-CHO. A 30 Hz and 150 pulses electrical stimulation, which had successfully induced adenosine releases from hippocampal slices (Yamashiro et al., 2017) showed no adenosine release in striatum slices (Fig2A). The failure of intense stimulation suggests the absence of electrically-induced adenosine release in the striatum. Meanwhile, adenosine releases were detected during -124 mOsm hypo-osmotic treatment, and releases quantified as the sum of area under the curve of $[Ca^{2+}]_i$ increases of sensor cells were 223.14±28.60% significantly larger in cocaine-treated wild type mice than in saline treated wild type mice (Fig. 2B). The increase of hypo-osmotically-induced adenosine release by cocaine withdrawal was occluded in $AQP4^{-/-}$ mice (Fig. 2C), suggesting the relation between the increase of AQP4 dependent adenosine release in the striatum and depressive-behavior.

The influence of AQP4-dependent adenosine increases on evoked-dopamine releases in the striatum.

The influence of adenosine on evoked-dopamine releases in the striatum was examined. An electrical stimulation of 30 Hz and 40 pulses induced $[Ca^{2+}]_i$ increases in the dopamine biosensor, D_2-CHO, and we quantified dopamine release as the sum of area under the curve of the $[Ca^{2+}]_i$ increase. Adenosine

significantly reduced dopamine release (10 μM, WT / Ado) to 61.92 ± 7.44% of control (WT / (-)) (Fig. 3A), while a non-selective adenosine receptor antagonist, 1 mM caffeine 146.67±23.50% significantly increased dopamine release (WT / Caffeine) to control (WT / (-)) (Fig. 3B). Furthermore, an A_1 antagonist, 10 μM DPCPX 156.32±17.39% significantly increased dopamine release (WT / DPCPX) to control (WT / (-)) (Fig. 3C), while an A_{2A} receptor antagonist, 50 nM SCH58261 did not change dopamine release (WT / SCH) difference from control (WT / (-)) (Fig. 3D). These results suggest that exogenous adenosine, as well as endogenous adenosine tone suppress evoked-dopamine release by A_1 receptor. The involvement of AQP4 in the adenosine tone, was examined by using AQP4$^{-/-}$ mice. As a result, dopamine release of AQP4$^{-/-}$ mice (AQP4$^{-/-}$) was 181.38±19.54% significantly larger than control (WT) (Fig. 3E). Moreover, DPCPX significantly reduced the dopamine release of AQP4$^{-/-}$ mice (AQP4$^{-/-}$ / DPCPX) to 54.70±8.49% of control(AQP4$^{-/-}$ / (-) (Fig. 3F). AQP4$^{-/-}$ mice showed larger dopamine release, and the enhanced dopamine release of in the presence of DPCPX was lacked in AQP4$^{-/-}$ mice. Thus, it was suggested that dopamine release is tonically suppressed by AQP4-dependent adenosine increase and subsequent A_1 receptor activation. The further suppression of dopamine release of AQP4$^{-/-}$ mice in the presence of DPCPX propose a facilitation of dopamine release by an A_1 receptor subpopulation, which is activated by adenosine release through other pathway rather than the AQP4 dependent pathway as will be discussed later in medial prefrontal cortex results.

Reduced dopamine release during depressive behavior

Dopamine releases in the striatum during depressive behavior was examined. The dopamine release of cocaine-treated mice (WT / Cocaine) was significantly smaller to 68.35±8.53% of control (WT /

(-)) (Fig. 4A). DPCPX (WT / Cocaine / DPCPX) 137.99 ± 18.11% significantly increased the dopamine release of cocaine-treated WT mice (WT / Cocaine / (-)) as in the case of saline-treated WT (Fig. 4B). These results suggests that the suppression of dopamine release by endogenous adenosine via A_1 receptor persists during depressive behavior. In contrast, the dopamine release of cocaine-treated $AQP4^{-/-}$ mice ($AQP4^{-/-}$ / Cocaine) was not significantly different from control ($AQP4^{-/-}$ / (-)) suggesting the ablation of reduced dopamine release by cocaine in $AQP4^{-/-}$ mice (Fig. 4C). These results indicate the lack of depressive behavior and associated reduction of dopamine release in $AQP4^{-/-}$ mice. In addition, DPCX did not change the dopamine release of cocaine-treated $AQP4^{-/-}$ mice ($AQP4^{-/-}$ / Cocaine / DPCPX and $AQP4^{-/-}$ / Cocaine / (-)) (Fig. 4D), indicating the loss of the A_1 receptor-mediated suppression of dopamine release in cocaine treated $AQP4^{-/-}$ mice. From these results, cocaine is supposed to induce depressive behavior by the increase of AQP4-dependent adenosine increase and subsequent suppression of dopamine release by A_1 receptors activation. Furthermore, A_1 receptor antagonists likely suppressed depressive behavior by restoring adenosine tone in the striatum.

Adenosine releases in mPFC

Since the altered neural activities in the mPFC is known to cause depressive behavior (Furuyashiki, 2012), the AQP4-dependent adenosine release in the mPFC was analyzed by using A_1-CHO. Both 150 pulses electrical stimulation at 30 Hz and -82.7 mOsM hypo-osmotic treatment induced adenosine releases in mPFC slices. The AUC after electrical stimulation did not show significant difference between $AQP4^{-/-}$ mice ($AQP4^{-/-}$) and control (WT) (Fig. 5A). In contrast, hypo-osmotically-induced adenosine release of $AQP4^{-/-}$ mice ($AQP4^{-/-}$) was 64.14 ± 9.24% significantly reduced to control (WT) (Fig. 5B). These results indicate that adenosine release in the mPFC is in

line with our previous study using hippocampal slices, where both electrical stimulation and hypo-osmotic treatment induce adenosine release and the hypo-osmotic release depends on AQP4 (Yamashiro et al., 2017). In order to determine the involvement of mPFC adenosine in depressive behavior, adenosine releases in cocaine-treated mice were examined. As results, both electrically and hypo-osmotically-induced adenosine release of cocaine-treated mice (WT / Cocaine) did not significant difference from those of control (WT / (-)) (Fig. 5C,D). Thus, it was suggested that these adenosine releases in the PFC were not involved in depressive behavior.

The involvement of adenosine in dopamine release in the mPFC

Since the decreased dopamine neurotransmission in the mPFC during depressive behavior is proposed (Furuyashiki, 2012), the involvement of adenosine in evoked-dopamine release in the mPFC was examined by using D_2-CHO. As in the striatum, the dopamine releases were induced by 40 pulses electrical stimulation at 30 Hz (arrow) in the mPFC. Exogenous adenosine 67.12 ± 8.13% significantly reduced dopamine release in the mPFC (WT / Ado) to control (WT / (-)) (Fig. 6A). Meanwhile, caffeine 66.96±5.82% significantly reduced in dopamine release in the mPFC (WT / Caffeine) to control (WT / (-)) (Fig. 6B). In addition, DPCPX 75.25±5.45% significantly reduced dopamine release (WT / DPCPX) to control (WT / (-)) (Fig. 6C), while SCH58261 did not change dopamine release (WT / SCH) to control (WT / (-)) (Fig. 6D). These results demonstrate the suppression of dopamine release in the PFC both by exogenous adenosine and A_1 receptor that the A_1 receptor activation by endogenous adenosine enhance dopamine release as in the opposite case in the striatum.

Downregulation of A_1 receptor in the mPFC during depressive behavior

Dopamine releases in the mPFC during depressive behavior were examined by D_2-CHO. The electrically induced dopamine releases in the mPFC slices from cocaine treated mice (WT / Cocaine) was not significantly different from control (WT / (-)) (Fig. 7A). This suggests the dopamine release in mPFC was not affected by the cocaine. However, DPCPX, which increased the dopamine releases in the mPFC of naïve mice, did not change the dopamine release in cocaine treated mice (WT / Cocaine / DPCPX and WT / Cocaine / (-)), suggesting the loss of the DPCPX induced increases of dopamine releases by cocaine (Fig. 7B). Since cocaine did not change adenosine release in the mPFC, the failure of DPCPX to change dopamine release in cocaine-treated mPFC is supposed to reflect downregulation of A_1 receptors. This point was examined in AQP4 AQP4$^{-/-}$ mice. Cocaine treatment did not change dopamine release in the mPFC of AQP4$^{-/-}$ mice ((AQP4$^{-/-}$ / (-) and AQP4$^{-/-}$ / Cocaine) (Fig. 7C). Meanwhile, DPCPX 61.26±5.27% significantly reduced dopamine release of cocaine-treated AQP4$^{-/-}$ mice (AQP4$^{-/-}$ / Cocaine / DPCPX) to control (AQP4$^{-/-}$ / Cocaine / (-)) (Fig. 7D). Hence, both the depressive behavior and the down-regulation of A_1 receptors were lost in AQP4$^{-/-}$ mice. All together, these results suggest that cocaine induces depressive behavior by down-regulating A_1 receptor in the mPFC through an AQP4-dependent pathway.

AQP4-dependent adenosine tone facilitates dopamine release in the mPFC.

The involvement of AQP4-dependent adenosine tone in evoked-dopamine release in the mPFC was examined by measuring the evoked-dopamine release of AQP4$^{-/-}$ mice using D_2-CHO. The dopamine release in the mPFC of AQP4$^{-/-}$ mice (AQP4$^{-/-}$) was not significantly different from wild type mice (WT) (Fig. 8A). The dopamine release in the mPFC of AQP4$^{-/-}$ mice in the presence of DPCPX, which

decrease the dopamine release in mPFC of WT mice was also not different from control ($AQP4^{-/-}$ / (-)) (Fig. 8B). These results indicate the lack of A_1 receptor-mediated reduction of dopamine release in the mPFC of $AQP4^{-/-}$ mice. Since hypo-osmotically-induced adenosine increase was smaller in the mPFC of $AQP^{-/-}$ mice, the loss of A_1 receptor-mediated reduction of dopamine release is likely due to the loss of A_1 receptor rather than the change of AQP4-dependent adenosine tone.

Two adenosine-dependent pathways regulating dopamine release.

The present discrepancy that both exogenous adenosine and DPCPX reduce dopamine release in mPFC was further examined. A_1 receptor is commonly involved in pre-synaptic inhibition, but the inhibition of A_1 receptor suppressed dopamine release in the mPFC. Pre-synaptic inhibition of dopamine release is mediated not only by A_1 receptor, but also other receptors, such as metabotropic glutamate receptor (*) or $GABA_A$ receptor (Yonezawa et al., 1998). Thus, one possibility is that a subpopulation of A_1 receptor inhibits glutamate or GABA release, which are simultaneously activated with dopamine release by electrical stimulation and pre-synaptically inhibit dopamine release.

To test this possibility, the effects of DPCPX in the presence of mGluR or $GABA_A$ receptors antagonist were examined. The effect of DPCPX was examined in the presence of $GABA_A$ receptor antagonist, 100 μM picrotoxin. In the presence of picrotoxin and DPCPX, the dopamine release (WT / PTX / DPCPX) was not significantly different from the control (WT / PTX / (-)) (Fig. 9A). Therefore, $GABA_A$ receptor antagonist occlude the effect of DPCPX. Since A_1 receptors are mostly resided in excitatory synapses rather than inhibitory ones (Chen et al., 2013), DPCPX supposed to affect on glutamatergic synapses. Since Group II metabotropic glutamate receptors (Group II mGluRs) regulate dopamine release in the mPFC and nucleus accumbens (Cartmell et al., 2000; Gupta and Young, 2018),

the possibility that DPCPX affects glutamatergic terminal involving Group II mGluR was examined.

The group II mGluR antagonist LY341495 was used to test this hypothesis and the dopamine release in the presence of 500 nM LY341495 and DPCPX and control (WT / LY / DPCPX and WT / LY / (-)) did not show significant difference (Fig. 9B). Hence, group II mGluR antagonist also occluded the effect of DPCPX on the dopamine release. If the DPCPX-sensitive glutamate release acts on ionotropic glutamate receptor and influences on dopaminergic neuron via neural circuit, AMPA receptor antagonist CNQX and NMDA receptor antagonist APV may affect. However, the dopamine release in the presence of DPCPX, together with 10 μM CNQX and 50 μM APV (WT / CNQX+APV / DPCPX) was 59.82 ± 4.12% significantly reduced to control (WT / CNQX+APV / (-)) (Fig. 9C), excluding the involvement of ionotropic glutamate receptors. These results indicate that the DPCPX affects the glutamate and GABA releases, which act on group II mGLuR or $GABA_A$ receptor locating at the presynaptic terminal releasing dopamine, respectively. Finally, the A_1 receptor down-regulation by cocaine treatment at dopamine release terminal, which is sensitive to exogenous adenosine in the mPFC. The dopamine release of cocaine treated mice in the presence of exogenous adenosine (WT / Cocaine / Ado) 73.79±6.82% significantly reduced to control (WT / Cocaine / (-)) (Fig. 9D). This result reflects that cocaine occluded the effect of DPCPX while did not influence on effect of exogenous adenosine. Overall, the dopamine release in the mPFC were regulated by two adenosine-dependent pathways, one pathways involving glutamate and GABA was down-regulated bycocaine treatment, while another direct adenosine inhibition of dopamine releasing terminal was not affected.

Discussion

In this study, we examined changes in adenosine and dopamine release associated with depression-like behavior using cocaine withdrawal as a model. Evaluation of depression-like behavior by the forced swimming test suggested that AQP4-mediated adenosine release may cause the development of depression-like behavior via A_1 receptors, because genetic disruption of AQP4 and A_1 receptor inhibition suppressed depression-like behavior. In the striatum, adenosine release upon electrical stimulation was not detected, and adenosine release was detected only by a greater osmotic pressure difference than in other brain regions, and disruption of the AQP4 gene had no effect on adenosine release. This indicates that the dynamics of extracellular adenosine in the striatum, where A_{2A} receptors are highly expressed, differs from that in the cortex and hippocampus. In contrast, slices of medial frontal cortex released adenosine upon electrical stimulation and hypoosmotic treatment, and the adenosine release upon hypoosmotic treatment was AQP4-dependent. This is consistent with two adenosine release pathways derived from neurons and astrocytes found in the hippocampus in a previous study (Yamashiro et al., 2017). In contrast, dopamine release by electrical stimulation was detected in both the striatum and medial frontal cortex. In the striatum, dopamine release was inhibited by adenosine, while it was enhanced by A_1 receptor inhibitors, and cocaine decreased dopamine release while increasing adenosine release. This suggests that in the striatum, cocaine enhances A_1 receptor activity by promoting adenosine release, resulting in decreased dopamine release. In contrast, in the medial frontal cortex, both adenosine and the A_1 receptor inhibitor inhibited dopamine release, and cocaine administration abolished the effect of the A_1 receptor inhibitor. $AQP4^{-/-}$ also abolished the effect of cocaine. Thus, in the medial frontal cortex, cocaine may deactivate A_1 receptors via an AQP4-mediated pathway, resulting in loss of endogenous adenosine modulation of dopamine release. Further investigation of the point at which A_1 receptor inhibitors suppressed dopamine release in the medial

frontal cortex, despite the fact that in many cases A_1 receptors are involved in presynaptic inhibition, suggests that glutamate release modulated by A_1 receptors may be mediated by GABAA receptors and gruop II mGluRs to inhibit dopaminergic, suggesting that A_1 receptors may inhibit dopaminergic release via GABAA receptors and gruop II mGluRs. Both striatum and medial frontal cortex showed modulation of dopaminergic neurotransmission associated with AQP4 and adenosine, both changes consistent with the onset of depressive-like behavior. However, because inhibition of A_1 receptors suppressed depressive-like behavior, the depressive-like behavior observed as a symptom of cocaine withdrawal may be attributed to a failure of the striatum, where adenosine release was increased and A_1 receptor activity was enhanced, rather than to the medial frontal cortex, where A_1 receptor dysfunction was observed.

The depression-like behavior due to cocaine withdrawal used in this study was quantified by administering cocaine to mice for 5 consecutive days, followed 14 days later by a forced swim test, using immobility time as an index. In preliminary experiments, immobility time did not increase after 7 days of cocaine administration. In $AQP4^{-/-}$ mice, cocaine-induced reduction of depressive behavior was observed. In the same mice, increased dopamine in the striatum measured by microdialysis and associated deficits in learning and motivation have been reported (Szu and Binder, 2016), consistent with the findings of this study that AQP4-mediated adenosine release inhibits dopamine neurotransmission consistent with the findings of this study that AQP4-mediated adenosine release inhibits dopamine neurotransmission. Acute administration of caffeine reduced cocaine-induced depression-like behavior. Caffeine has already been shown to have antidepressant effects, as it has been reported to decrease immobility time in a forced swimming test in normal mice (Szopa et al., 2016).The fact that acute administration of DPCPX decreased cocaine-induced depressive-like

behaviors, as well as caffeine, suggests that caffeine is a inhibited depressive-like behavior by blocking A_1 receptors. Similar to caffeine, DPCPX has also been reported to decrease immobility time in the forced swim test in normal mice (Szopa et al., 2018). Therefore, it is possible that the effects of caffeine and DPCPX on depression-like behavior in the present study may not have inhibited the changes induced by cocaine.

No adenosine release associated with electrical stimulation was detected in the striatum. This result is consistent with reports of very low detection success rates when similar measurements are made with fast scan cyclic voltammetry (Pajski and Venton, 2013). On the other hand, there have been several reports of detection of adenosine associated with electrical stimulation when adenosine in the striatum of individual animals is measured by microdialysis (Cechova and Venton, 2008; Cechova et al., 2010). A possible reason for the loss of adenosine release associated with electrical activity when sliced could be that blood flow is required for adenosine release via AQP4. In vivo, glucose consumption increases with neural activity, resulting in increased glucose uptake from capillaries via glucose transporters in astrocytes and glycogen degradation within astrocytes. These increases in glucose within astrocytes are thought to increase osmotic pressure, leading to increased water absorption via AQP4 localized at the site of contact between astrocytes and blood vessels, resulting in cell swelling and adenosine release. In contrast, in slices, glucose and water influx from capillaries to astrocytes did not occur because neurons were able to take up glucose directly from extracellular fluid. Adenosine release from the striatum by hypo-osmotic treatment was not induced at -82.7 mOsM, which is effective in the frontal cortex, but required a concentration of -120 mOsM and was not affected by disruption of the AQP4 gene. Cocaine, on the other hand, increased this adenosine release, but whether this release is dependent on AQP4 has not yet been examined. It is quite possible that

cocaine increased AQP4-dependent adenosine release.

In the striatum, exogenous adenosine inhibited dopamine release, while A_1 receptor inhibitors enhanced dopamine release. In addition, $AQP4^{-/-}$ showed increased dopamine release. These results strongly suggest that adenosine released in an AQP4-dependent manner in the striatum constanly inhibits dopamine release. Presynaptic inhibition by A_1 receptors on dopaminergic nerve endings has been reported in the striatum, supporting the above results (Yabuuchi et al., 2006). It has also been reported that blockers of A_{2A} receptors do not affect dopamine release in the striatum, consistent with our results (Ross and Venton, 2015). On the other hand, we found a decrease in DPCPX-induced dopamine release in $AQP4^{-/-}$ striatum. This suggests that adenosine released independently of AQP4 may facilitate dopamine release. It is highly likely that AQP4-independent adenosine plays a major role in the altered neural activity in $AQP4^{-/-}$, especially since no difference in adenosine release between wild-type and $AQP4^{-/-}$ mice was observed.

Dopamine release in the medial frontal cortex was inhibited by exogenous adenosine, and this phenomenon was not affected by cocaine. On the other hand, A_1 receptor inhibitors also suppressed dopamine release, and this phenomenon was abolished by cocaine, suggesting the involvement of $GABA_A$ receptors and group II mGluRs in the A_1 receptor suppression, but the mechanism of action of exogenous adenosine is not clear. Future studies are planned to examine the effects of ion-channel glutamate receptor or $GABA_A$ receptor inhibitors on adenosine-induced dopamine release. If these inhibitors have no effect, then adenosine is acting directly on dopamine nerve endings. In that case, only A_1 receptors on glutamatergic nerve endings would be activated by endogenous adenosine and deactivated by cocaine. The A_1 receptors of dopaminergic nerve endings would not be deactivated by cocaine in either the striatum or medial frontal cortex, whereas they would be activated by endogenous

adenosine in the striatum. Analysis of the fine structure of nerve endings and receptor localization would be necessary to test these possibilities.

In AQP4$^{-/-}$, dopamine release in the medial frontal cortex was the same as in wild-type mice, but in these mice, the A_1 receptor inhibitor abolished the reduction in dopamine release; the differential effects of AQP4$^{-/-}$ and A_1 receptor inhibitors also need to be examined in the future. -/-, it is quite possible that these neuronal circuits were affected as a result of decreased endogenous adenosine and increased glutamate and dopamine release. In this sense, the effects of inhibition of $GABA_A$ receptors and group II mGluRs in AQP4$^{-/-}$ should be examined.

In the medial frontal cortex, cocaine did not affect adenosine release by electrical stimulation or hypoosmotic treatment, nor dopamine release, but the inhibition of dopamine release by DPCPX was abolished. This suggests that depressive-like behavior may be related to A_1 receptor dysfunction rather than adenosine release. AQP4$^{-/-}$, which did not exhibit cocaine-induced depressive-like behavior, also showed no change in dopamine release, and DPCPX suppressed dopamine release as in normal mice. This suggests that cocaine causes a reduction in A_1 receptor function in the medial frontal cortex that is associated with depression-like behavior, and that this reduction is abolished in AQP4$^{-/-}$. In this connection, deep brain stimulation (Bekar et al., 2008) and electroconvulsive therapy (Sadek et al., 2011) have been reported to show antidepressant effects by inducing increased expression of A_1 receptors in the cortex. In the hippocampus, it has also been reported that increased A_1 receptors increase expression of Homer1a and promote resistance to depression (Serchov et al., 2015). In the nucleus accumbens, A_1 receptor expression decreases during cocaine withdrawal (Toda et al., 2003), and a similar mechanism is expected in the cortex. In other words, cocaine may induce depressive-like behavior by decreasing A_1 receptors through some pathway via adenosine release via AQP4 in

astrocytes.

In the medial frontal cortex, dopaminergic innervation from the ventral tegmental area mainly inputs deep, while glutamatergic innervation mainly inputs near the surface (Van Eden et al., 1987; Jay et al., 1992; Kuroda et al., 1998), and GABAergic inter neurons mediate both (Yonezawa et al., 1998; Zhu et al., 2004). This suggests a glutamate-GABA-dopamine regulatory mechanism by endogenous adenosine. It has also been reported that continuous cocaine administration in the medial frontal cortex downregulates the expression of group II mGluRs (Huang et al., 2007), and the fact that the action of DPCPX was abolished by inhibitors of group II mGluRs suggests that cocaine suppressed the expression of group II mGluRs This study has demonstrated that cocaine abstinence is a key factor in the development of the DPCPX receptor, and that cocaine suppresses the expression of group II mGluRs.

The present study demonstrates that cocaine withdrawal-induced depressive-like behavior is accompanied by a suppression of dopamine release via A_1 receptors due to increased adenosine release associated with hypoosmotic processing in the striatum and dysfunction of A_1 receptors in the medial frontal cortex. Future work is needed to analyze the molecular mechanisms of AQP4 in the striatum and A_1 receptors in the medial frontal cortex, including their expression and functional regulation. It has also been reported that adenosine release pathways differ among brain regions (Pajski and Venton, 2013), and detailed studies on the association between AQP4-independent adenosine and dopamine release will be necessary in the future. While currently widely used antidepressants mainly inhibit serotonin reuptake and increase extracellular serotonin concentration, this study suggests the possibility of a new antidepressant therapy based on dopamine and adenosine regulation. In recent years, a link between neuroinflammation and psychiatric disorders such as depression has been

proposed, and it is quite possible that the activation of astrocytes associated with stress or drug-induced inflammation and the anti-inflammatory effects of adenosine may contribute to the pathogenic process. We will continue to investigate the relationship between glial cell activation and adenosine and dopamine signaling in various brain regions.

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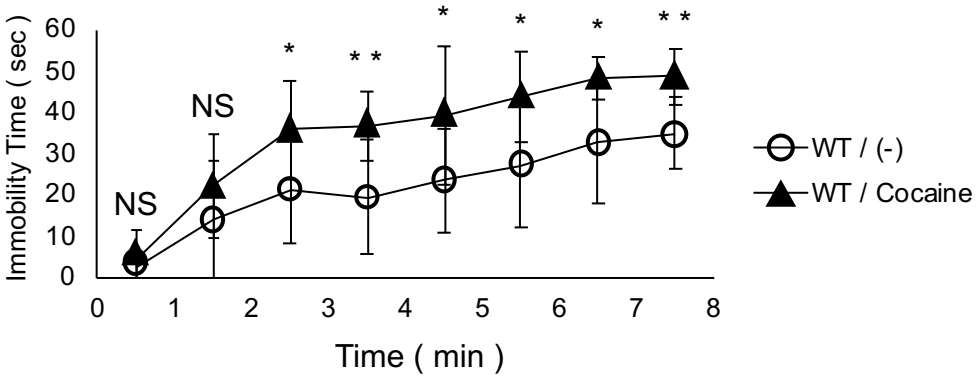
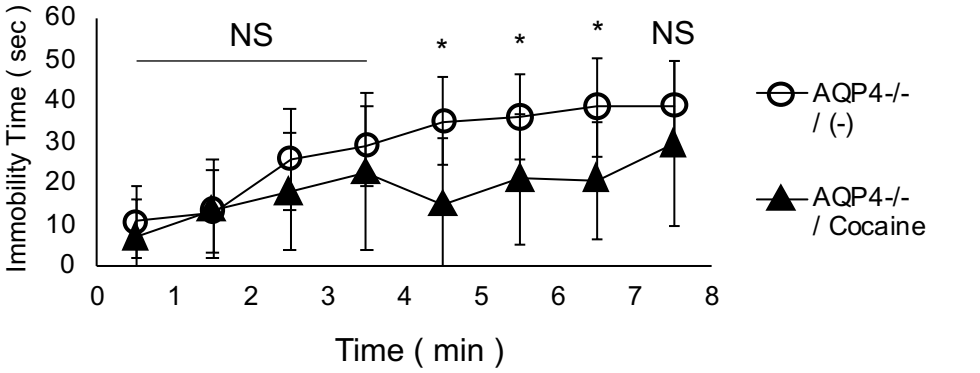
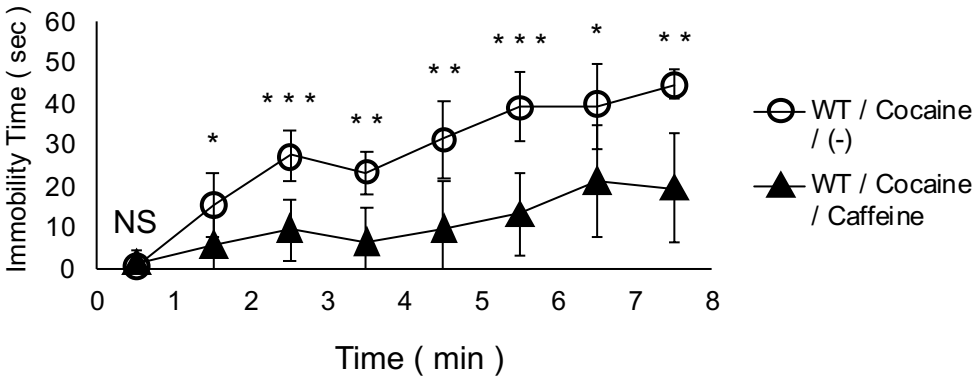
A.


Immobility Time (sec)
Time (min)
NS
NS
WT / (-)
WT / Cocaine

B.


Immobility Time (sec)
Time (min)
NS
NS
AQP4-/- / (-)
AQP4-/- / Cocaine

C.


Immobility Time (sec)
Time (min)
NS
WT / Cocaine / (-)
WT / Cocaine / Caffeine

D.

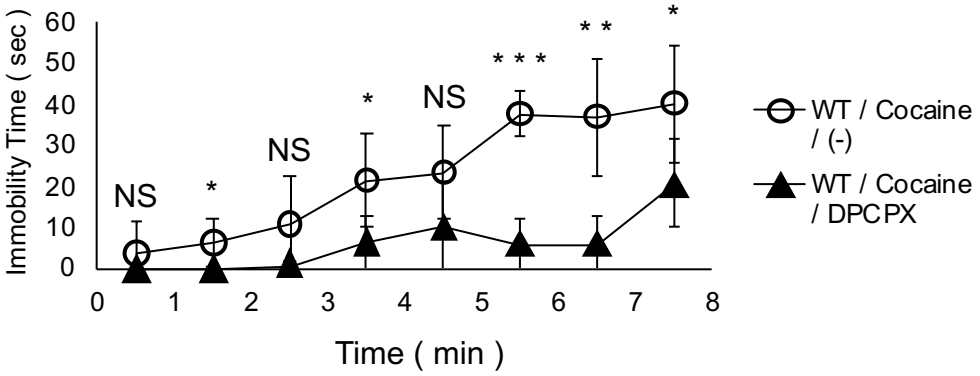

Immobility Time (sec)
Time (min)
NS
NS
NS
WT / Cocaine / (-)
WT / Cocaine / DPCPX

Fig. 1 Depressive behavior of cocaine-treated mice were suppressed by adenosine receptor antagonists or in AQP4$^{-/-}$ mice. Depressive behavior during cocaine withdrawal was assessed by the time-dependent changes of immobility time in forced swim test and the influences of pharmacological or genetical interventions were examined. A. Cocaine induced depressive behavior. Wild type mice were treated with saline (WT / (-), n = 8) or cocaine (WT/Cocaine, n=8). Saline or cocaine were treated as described in Materials and Method for inducing depressive behavior during cocaine withdrawal. B. Depressive behaviors of AQP4$^{-/-}$ mice. AQP4$^{-/-}$ mice were treated with saline (AQP4$^{-/-}$ / (-), n = 6) or cocaine (AQP4$^{-/-}$ / cocaine, n = 7). C. The effect of a non-selective adenosine receptor antagonist, caffeine. Saline (WT / Cocaine / (-), n = 6) or 50 mg/kg caffeine (WT / Cocaine / Caffeine, n = 7) were treated 40 min prior to forced swim test. D. The effect of an A_1 adenosine receptor selective antagonist DPCPX. Saline (WT / Cocaine / (-), n = 5) or 4 mg/kg DPCPX (WT / Cocaine / DPCPX, n = 5) were treated 30 min prior to forced swim test. Mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS, not significant.

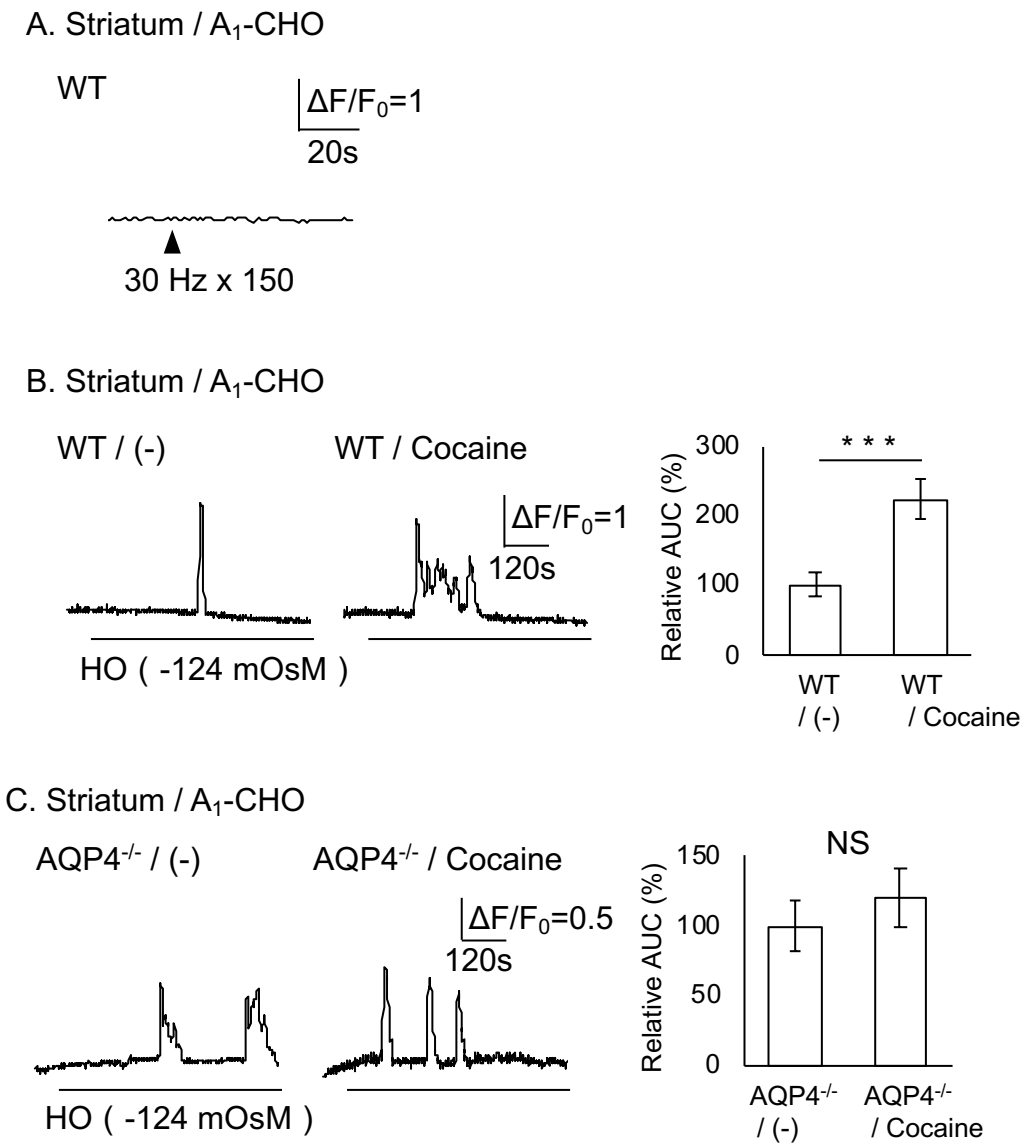


Fig. 2 Increased adenosine release in the striatum during depressive behavior. Adenosine from striatum slices were measured by A_1-CHO (Striatum / A_1-CHO). A. Representative time dependent changes of $[Ca^{2+}]_i$ during electrical stimulation (arrowhead, 30 Hz, 150 pulses) to slices prepared from wild type mice (WT). B. Adenosine releases from slices of saline- (WT / (-), n = 6 slices) or cocaine-treated wild type mice (WT / cocaine, n = 6 slices) during hypo-osmotic treatment. Representative time dependent changes of $[Ca^{2+}]_i$ of A_1-CHO (left, center). Hypo-osmotic treatment

(HO; -124 mOsM) were indicated by lines. $[Ca^{2+}]_i$ increase during HO treatment were shown as relative area under the curves (AUC, right) normalized by control. C. Adenosine releases from slices of saline ($AQP4^{-/-}$ / (-), n = 6 slices) or cocaine ($AQP4^{-/-}$ / Cocaine, n = 6 slices) treated $AQP4^{-/-}$ mice during hypo-osmotic treatment. Mean ± SE, *** $p<0.001$, NS, not significant.

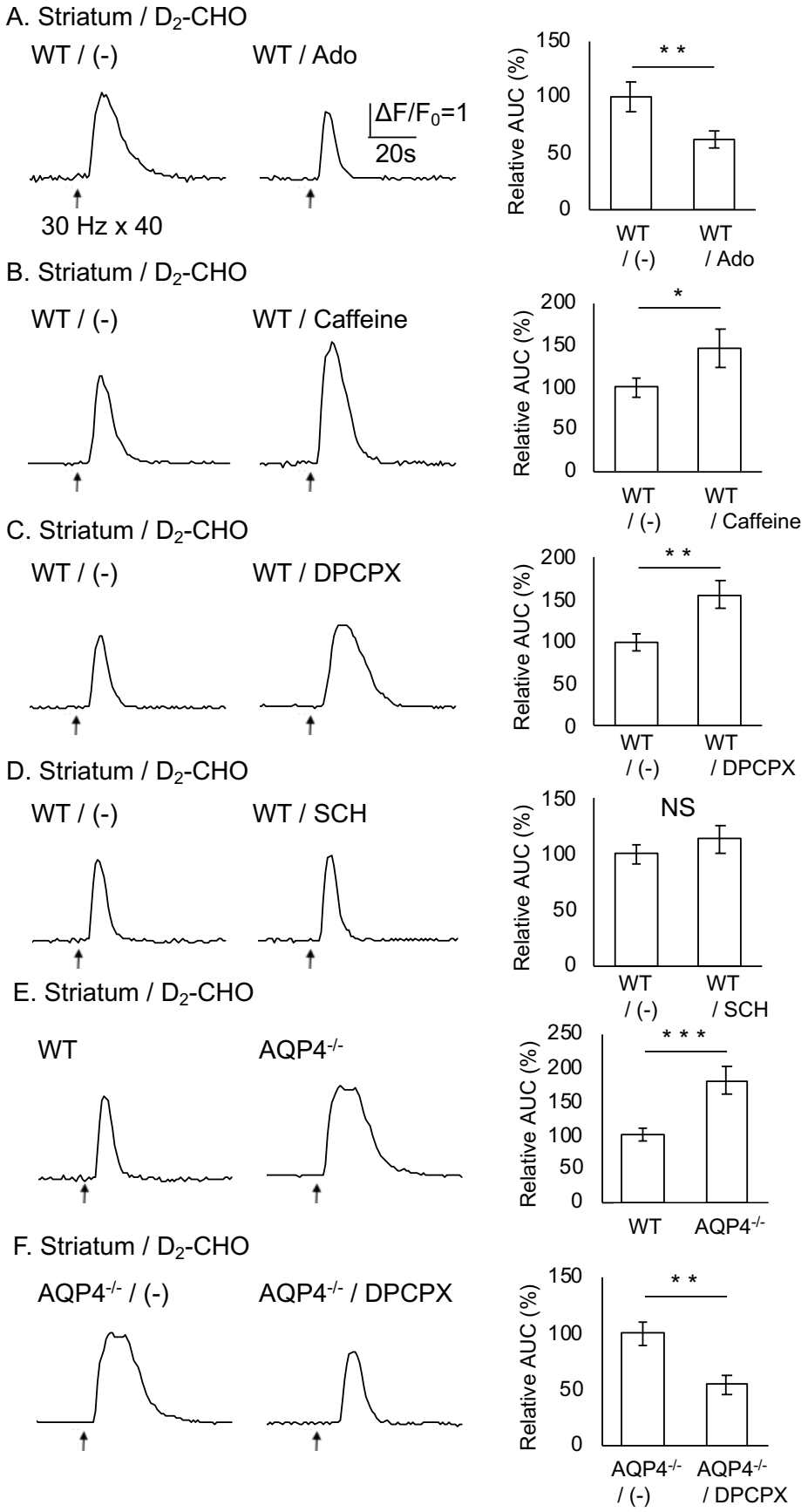

A. Striatum / D2-CHO
WT / (-)
WT / Ado
ΔF/F0=1
20s
30 Hz x 40
Relative AUC (%)
150
100
50
0
* *
WT / (-)
WT / Ado
B. Striatum / D2-CHO
WT / (-)
WT / Caffeine
Relative AUC (%)
200
150
100
50
0
*
WT / (-)
WT / Caffeine
C. Striatum / D2-CHO
WT / (-)
WT / DPCPX
Relative AUC (%)
200
150
100
50
0
* *
WT / (-)
WT / DPCPX
D. Striatum / D2-CHO
WT / (-)
WT / SCH
Relative AUC (%)
150
100
50
0
NS
WT / (-)
WT / SCH
E. Striatum / D2-CHO
WT
AQP4-/-
Relative AUC (%)
250
200
150
100
50
0
* * *
WT
AQP4-/-
F. Striatum / D2-CHO
AQP4-/- / (-)
AQP4-/- / DPCPX
Relative AUC (%)
150
100
50
0
* *
AQP4-/- / (-)
AQP4-/- / DPCPX

Fig. 3 Suppression of evoked-dopamine release in the striatum by AQP4 dependent increase of adenosine. Evoked-dopamine releases in striatal slices were measured by D_2-CHO (Striatum / D_2-CHO). A. The effect of adenosine. Slices from wild type mice (WT) were electrically stimulated (30 Hz, 40 pulses) in the absence (WT / (-), n = 4 slices) or presence of 10 μM adenosine (WT / Ado, n = 5 slices). Representative time dependent changes of $[Ca^{2+}]_i$ of D_2-CHO (left, center) during electrical stimulation (arrow). $[Ca^{2+}]_i$ increases of D_2-CHO for one minute after stimulation were shown as relative AUC (right) normalized by control. B. The effect of caffein. Evoked-dopamine release in the absence (WT / (-), n = 6 slices) or presence of 1 mM caffein (WT / Caffeine, n = 6 slices). C. The effect of DPCPX. Evoked-dopamine release in the absence (WT / (-), n = 6 slices) or presence of 10 μM DPCPX (WT / DPCPX, n = 4 slices). D. The effect of an A_{2A} selective antagonist SCH58261. Evoked-dopamine release in the absence (WT / (-), n = 6 slices) or presence of 10 μM DPCPX (WT / DPCPX, n = 4 slices). E. The involvement of AQP4. Evoked-dopamine releases in wild type (WT, n = 6 slices) and AQP4 knock out mice ($AQP4^{-/-}$, n = 7 slices). F. The effect of DPCPX on $AQP^{-/-}$ mice. Evoked-dopamine releases in the absence ($AQP4^{-/-}$ / (-), n = 7 slices) or presence of DPCPX ($AQP4^{-/-}$ / DPCPX, n = 5 slices). Mean ± SE. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS, not significant, t-test.

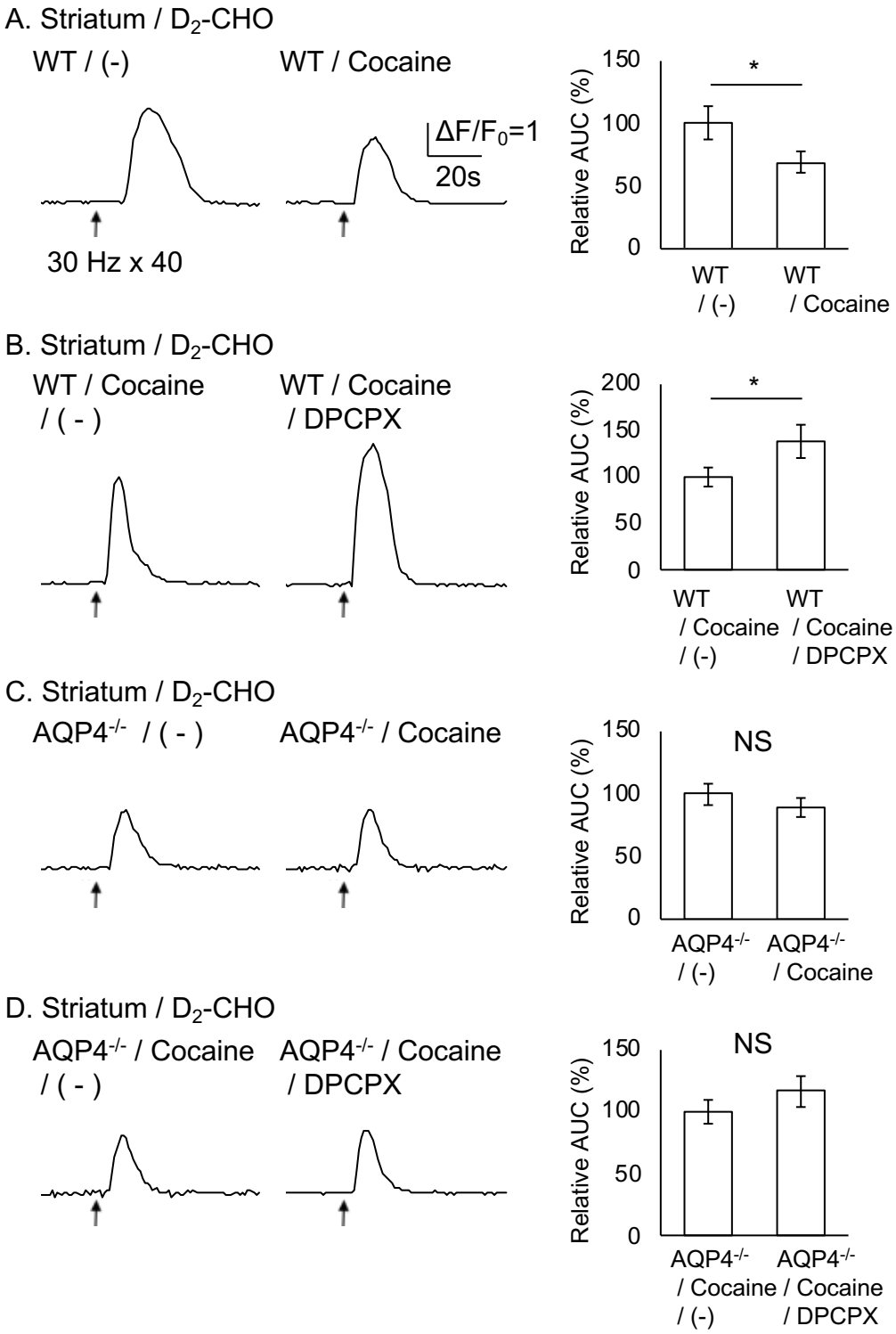


Fig. 4 Reduction of evoked-dopamine release in the striatum of mice during depressive behavior and its reversal in AQP4 knock out mice. Dopamine releases in striatal slices were

measured by D_2-CHO (Striatum / D_2-CHO). A. The effect of cocaine. Slices from wild type mice (WT) with (WT / cocaine, n = 7 slices) or without cocaine treatment (WT / (-), n = 10 slices) were electrically stimulated (30 Hz, 40 pulses). B. The effect of DPCPX in cocaine treated mice. Evoked-dopamine release in the absence (WT / Cocaine / (-), n = 6 slices) or presence of 1 mM caffein (WT / Cocaine / DPCPX, n = 5 slices). C. The effect of cocaine on $AQP4^{-/-}$ mice. Evoked-dopamine releases in $AQP4^{-/-}$ mice with ($AQP4^{-/-}$ / cocaine, n = 9 slices) or without cocaine treatment ($AQP4^{-/-}$ / (-), n = 9 slices). D. The effect of DPCPX in cocaine-treated $AQP4^{-/-}$ mice. Evoked-dopamine release in cocaine-treated $AQP4^{-/-}$ mice in the absence ($AQP4^{-/-}$ / Cocaine /(-), n = 7 slices) or presence of cocaine ($AQP4^{-/-}$ / Cocaine / DPCPX, n = 6 slices). Mean ± SE. * $p<0.05$, NS, not significant, t-test.

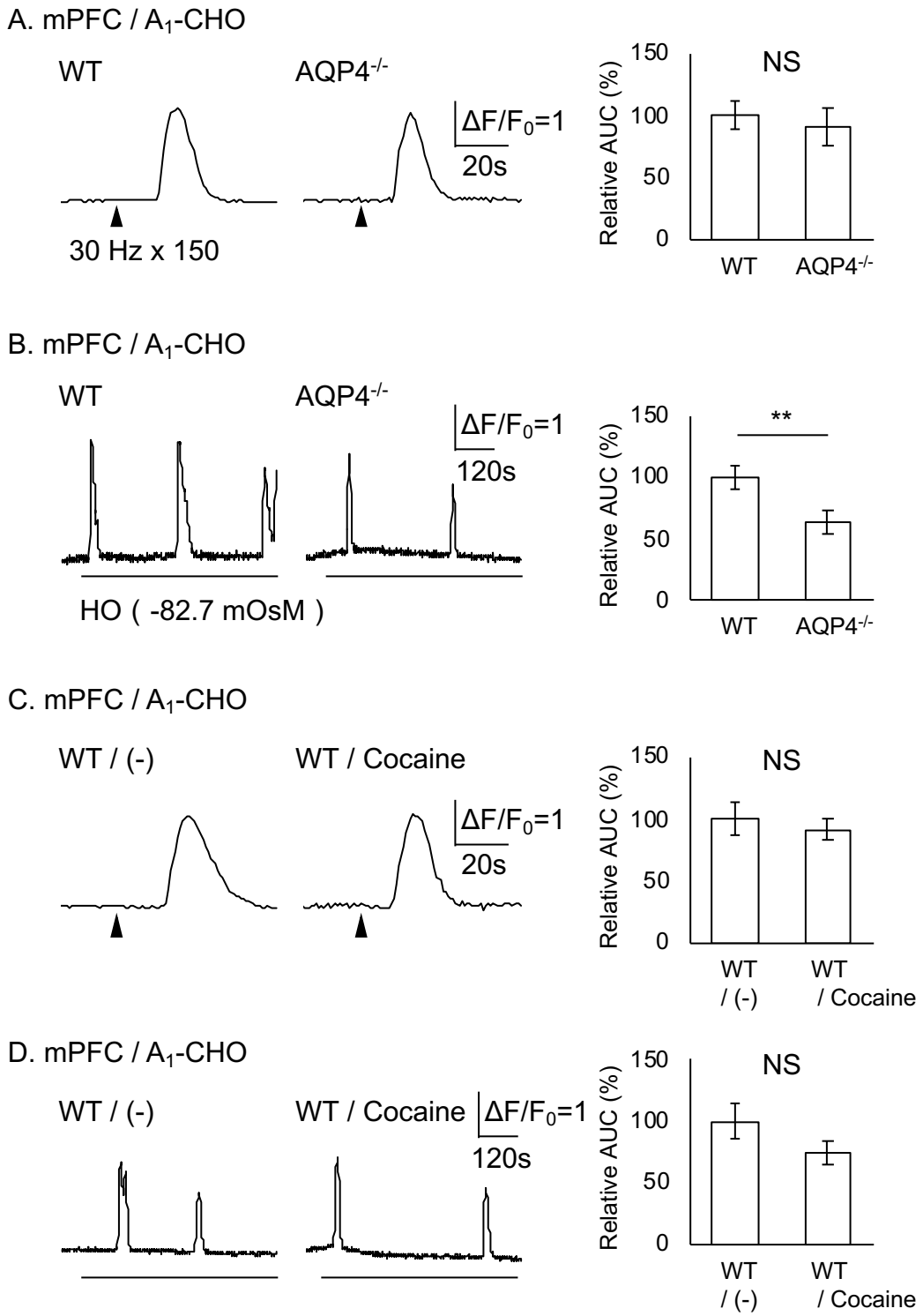


Fig. 5 Adenosine release in mPFC is not associated with depressive behavior. Adenosine from medial prefrontal cortex (mPFC) slices were measured by A_1-CHO (mPFC / A_1-CHO). A. Evoked-

adenosine releases from wild type (WT, n = 6 slices) and AQP4$^{-/-}$ mice (AQP4$^{-/-}$, n = 5 slices). Representative time dependent changes of $[Ca^{2+}]_I$ of A_1-CHO (left, center) during electrical stimulation (150 pulses at 30 Hz, arrowhead). $[Ca^{2+}]_i$ increases of A_1-CHO for one minute after stimulation were shown as relative AUC (right) normalized by control. B. Slices from wild type mice (WT, n = 6 slices) and AQP4$^{-/-}$ mice (AQP4$^{-/-}$, n = 6 slices) were hypo-osmotically stimulated (HO; -82.6 mOsM) as indicated by lines. C. Evoked-adenosine releases in the absence (WT / (-), n = 7 slices) or presence of cocaine (WT / Cocaine, n = 6 slices. D. Hypo-osmotically-induced adenosine release in mice with (WT / cocaine, n = 6 slices) or without cocaine treatment (WT / (-), n = 6 slices). Mean ± SE, ** $p<0.01$, NS, not significant.

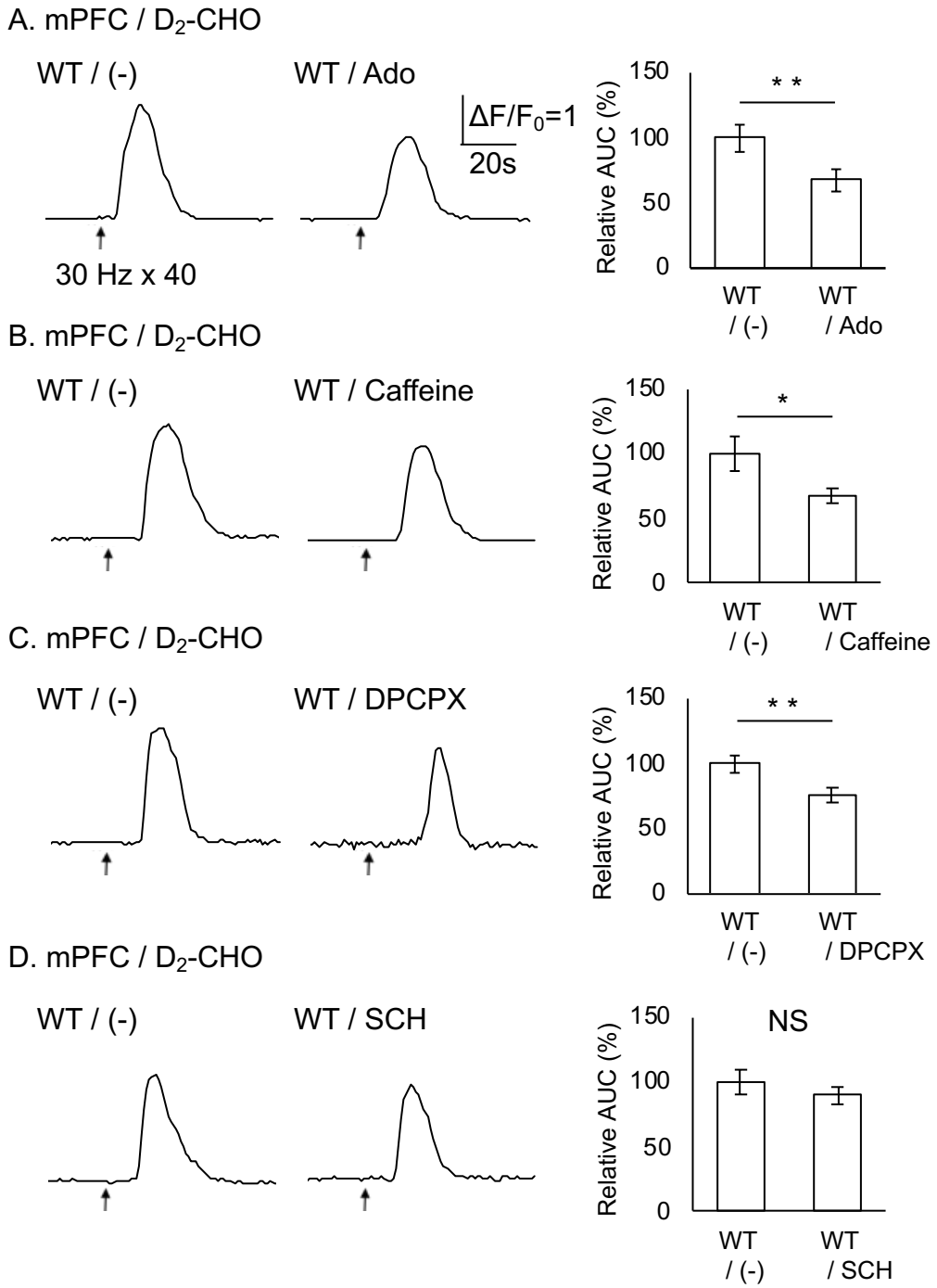


Fig. 6 Suppression of evoked-dopamine release in the mPFC by adenosine. Dopamine from mPFC slices were measured by D_2-CHO (mPFC / D_2-CHO). A. The effect of adenosine. Slices from wild type mice (WT) were electrically stimulated (30 Hz, 40 pulses) in the absence (WT / (-), n = 8 slices) or presence of adenosine (WT / Ado, n = 9 slices). B. The effect of caffein. Evoked-dopamine

releases in the absence (WT / (-), n = 8 slices) or presence of caffein (WT / Caffeine, n = 8 slices). C. The effect of DPCPX. Evoked-dopamine releases in the absence (WT / (-), n = 8 slices) or presence of DPCPX (WT / DPCPX, n = 9 slices). D. The effect of SCH58261. Evoked-dopamine releases in the absence (WT / (-), n = 10 slices) or presence of SCH58261 (WT / SCH, n = 10 slices). Mean ± SE. * $p<0.05$, ** $p<0.01$, NS, not significant, t-test.

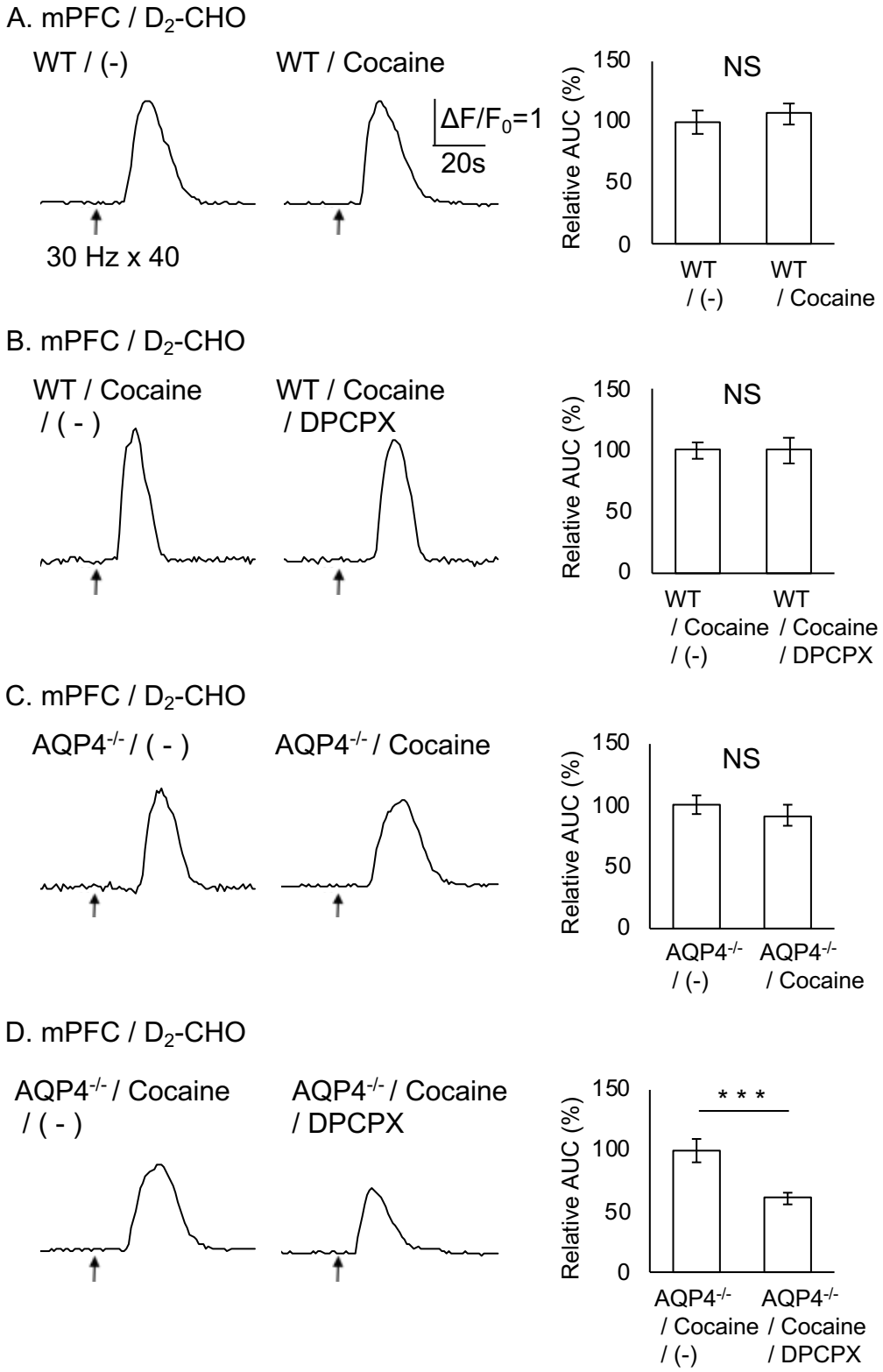


Fig. 7 Downregulation of A_1 receptor in the mPFC of cocaine-treated mice. Dopamine from mPFC slices were measured by D_2-CHO (mPFC / D_2-CHO). A. The effect of cocaine. Slices from

wild type mice (WT) with (WT / cocaine, n = 12 slices) or without cocaine treatment (WT / (-), n = 9 slices) were electrically stimulated (30 Hz, 40 pulses). B. The effect of DPCPX in cocaine treated mice. Evoked-dopamine releases in the absence (WT / Cocaine / (-), n = 8 slices) or presence of DPCPX (WT / Cocaine / DPCPX, n = 8 slices). C. The effect of cocaine in $AQP4^{-/-}$ mice. Evoked-dopamine releases in absence ($AQP4^{-/-}$ / (-), n = 9 slices) or presence of cocaine ($AQP4^{-/-}$ / cocaine, n = 8 slices). D. The effect of DPCPX in cocaine treated $AQP4^{-/-}$ mice. Evoked-dopamine releases in the absence ($AQP4^{-/-}$ / Cocaine /(-), n = 8 slices) or presence of DPCPX ($AQP4^{-/-}$ / Cocaine / DPCPX, n = 7 slices). Mean ± SE. * $p<0.05$, NS, not significant, t-test.

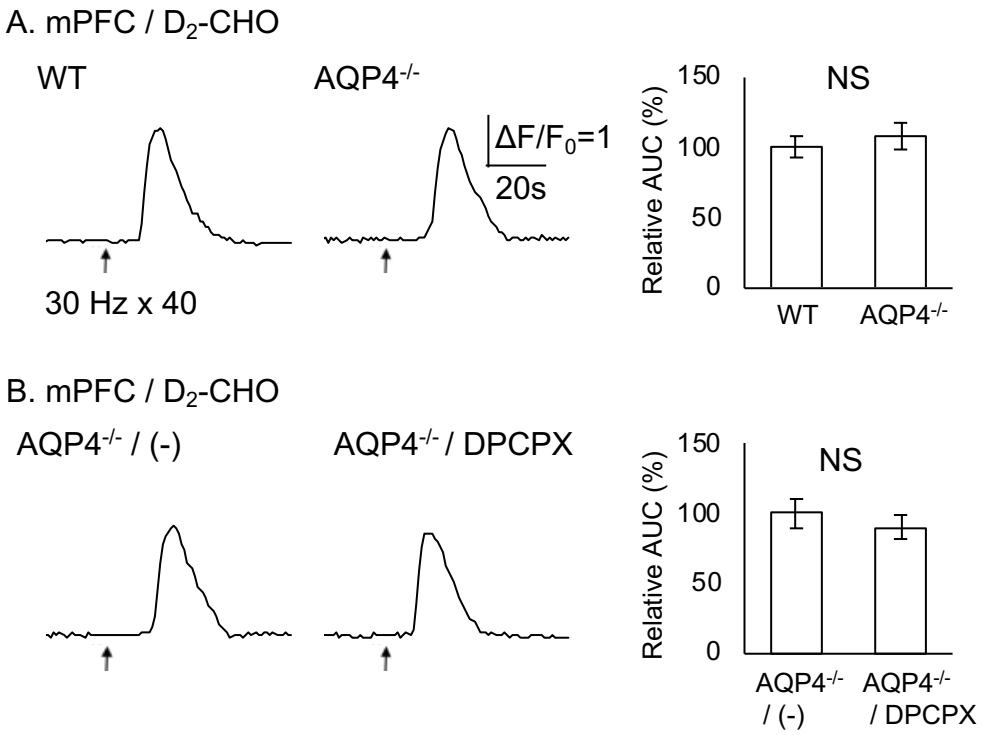


Fig. 8 Facilitation of evoked-dopamine release in the mPFC by AQP4-dependent increase of adenosine. Evoked-dopamine release from mPFC slices were measured by D_2-CHO (mPFC / D_2-CHO). A. The involvement of AQP4. Slices from wild type mice (WT, n = 7 slices) and AQP4 knock out mice ($AQP4^{-/-}$, n = 7 slices) were electrically stimulated (30 Hz, 40 pulses). B. The effect of DPCPX on $AQP4^{-/-}$ mice. Evoked-dopamine releases in the absence ($AQP4^{-/-}$ / (-), n = 7 slices) or presence of DPCPX ($AQP4^{-/-}$ / DPCPX, n = 8 slices). NS, not significant, t-test.

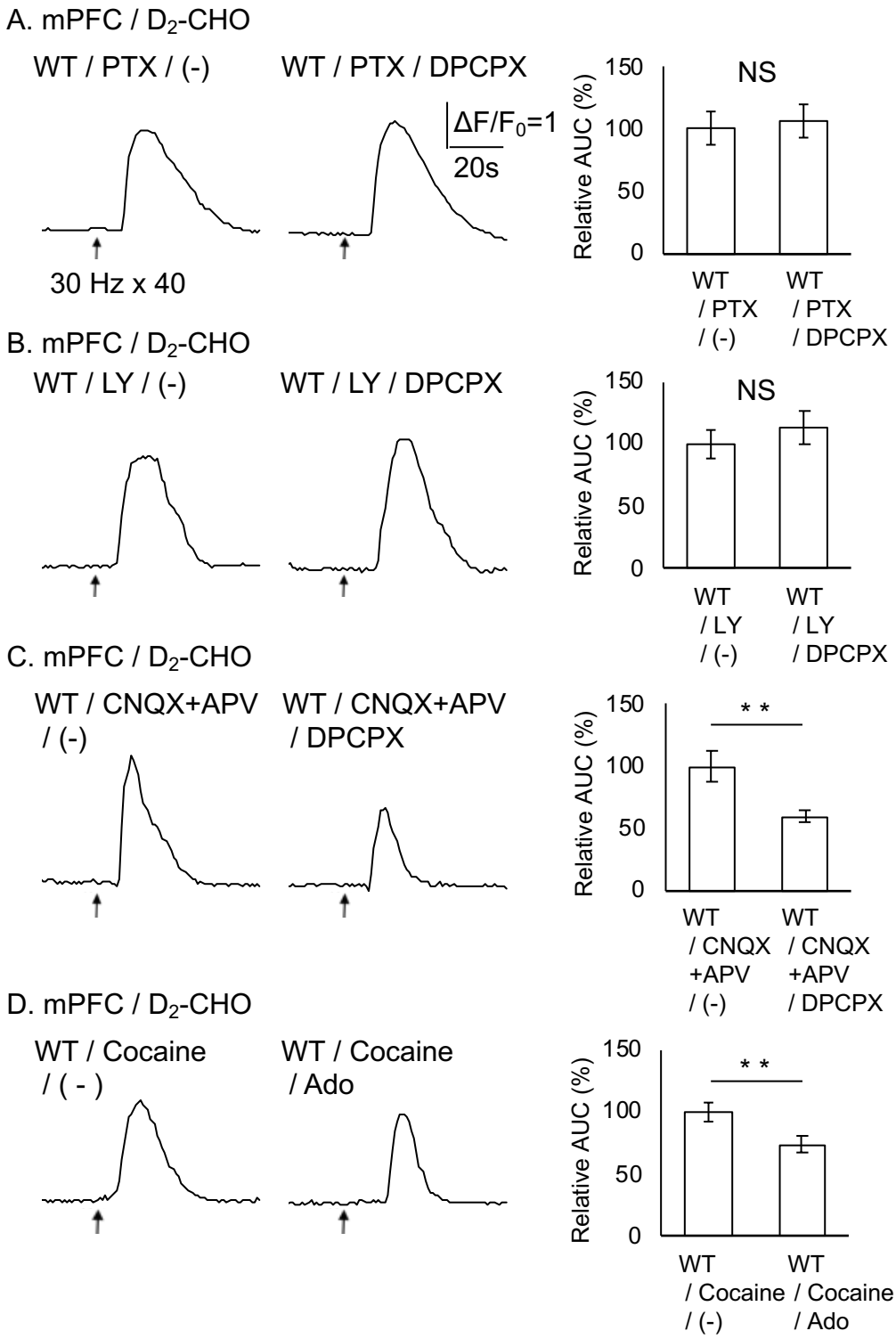


Fig. 9 Dual regulation of dopamine release in the mPFC by adenosine tone. Evoked-dopamine releases from mPFC slices were measured by D_2-CHO (mPFC / D_2-CHO). A. The effect of DPCPX

in the presence of a GABA receptor antagonist picrotoxin, PTX. Slices from wild type mice (WT) were electrically stimulated (30 Hz, 40 pulses) in the presence of 100 μM PTX and DPCPX (WT / PTX / DPCPX, n = 6) or PTX alone (WT / PTX / (-), n = 6 slices). B. The effect of Group II mGluR antagonist LY341495 (LY). Evoked-dopamine release int the presence of 500 nM LY and DPCPX (WT / LY / DPCPX, n = 8 slices) or LY alone (WT / LY / (-), n = 8 slices). C. The effect of ion channel type glutamate receptor antagonists, CNQX and APV. Evoked-dopamine releases in the presence of 10 μM CNQX, 50 μM APV and DPCPX (WT / CNQX+APV / DPCPX, n = 9 slices) or CNQX+APV alone (WT / CNQX+APV / (-), n = 10 slices). D. The effect of adenosine on cocaine-treated mice. Evoked-dopamine releases in slices from cocaine-treated wild type mice in the absence (WT / Cocaine / (-), n = 9 slices) of presence of adenosine (WT / Cocaine / Ado, n = 9 slices). NS, not significant, t-test