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# Functional role of the endocannabinoid system and AMPA/kainate receptors in 5-$HT_{2A}$ receptor-mediated wet dog shakes

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**Abstract**

These experiments sought to determine the influence of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors and the endocannabinoid system in the functional expression of the serotonin (5-HT) type 2A receptor-mediated wet dog shake response. Male Long–Evans rats were pretreated with either 1 mg/kg i.p. of the 5-$HT_{2A/2C}$ receptor antagonist ketanserin; 1, 10 or 30 mg/kg i.p. of the AMPA/kainate antagonist 6,7-dinitroquinnoxaline-2,3-dione (DNQX); 1, 5 or 10 mg/kg i.p. of the endocannabinoid uptake inhibitor AM404; or 1, 5 or 10 mg/kg i.p. of the cannabinoid $CB_1$ receptor antagonist AM 251 prior to injection of the 5-$HT_{2A/2C}$ receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI, 1 mg/kg i.p.). Results demonstrated that 10 mg/kg of AM404 significantly reduced the expression of DOI-induced wet dog shakes, but lower doses were ineffective. Administration of AM251 did not induce wet dog shakes behavior when administered alone, but significantly potentiated DOI-induced wet dog shaking behavior at a dose of 10 mg/kg. Pretreatment with DNQX significantly reduced the expression of DOI-induced wet dog shakes at all doses tested. These data suggest that AMPA/kainate receptors play a role in the mediation of 5-$HT_{2A}$ receptor activity, whereas the endocannabinoid system may act as a regulatory buffer system during periods of elevated activity, but not under basal conditions.


*Keywords:* 5-$HT_{2A}$; Endocannabinoid; Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA); Kainate; Wet dog shake; (Rat)

## 1. Introduction

The serotonin (5-HT) type 2 receptor family of receptors is one of several families of 5-HT receptors, which are metabotropic, G-protein coupled receptors (Barnes and Sharp, 1999). The 5-$HT_2$ family is divided into three subtypes: the 5-$HT_{2A}$, 5-$HT_{2B}$ and 5-$HT_{2C}$ (Barnes and Sharp, 1999). The 5-$HT_{2A}$ receptor is of increasing interest to behavioral neuroscience because of accumulating evidence implicating it in the pathophysiology and pharmacotherapy of several emotional and eating disorders (e.g., de Angelis, 2002).

Administration of the 5-$HT_{2A/2C}$ receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) elicits a characteristic behavioral response that is manifested as an increase in wet dog shakes in rats or the head twitch response in mice (e.g., Gorzalka and Hanson, 1998; Darmani and Ahmad, 1999). Research indicates that the wet dog shake response is mediated by selective activation of 5-$HT_{2A}$ receptors (Yap and Taylor, 1983; Schreiber et al., 1995), and wet dog shakes have proved to be a useful, reliable and non-invasive gauge of alterations in endogenous 5-$HT_{2A}$ receptor activity (Yap and Taylor, 1983; Watson and Gorzalka, 1990). For example, chronic administration of antidepressants has been shown to lead to a reduction in DOI-induced wet dog shakes, which correlates with a downregulation of central 5-$HT_{2A}$ receptors (Goodwin et al., 1984). Both wet dog shakes in rats and head twitches in mice have been employed in studies on the regulation of 5-$HT_{2A}$ receptors. Studies employing these behavioral assays have helped to determine that the $\alpha_2$ adrenoreceptor, NMDA glutamatergic receptor and 5-$HT_{1A}$ receptor all exert negative regulation over 5-

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$HT_{2A}$ receptor-mediated responses (Darmani et al., 1990; Kim et al., 1998; Matsumoto et al., 1997).

The cannabinoid system is believed to interact with serotonergic activity and specifically the 5-$HT_{2A}$ receptor. Administration of cannabinoid $CB_1$ receptor ligands in vitro has been shown to reduce both electrically induced and $Ca^{2+}$ induced 5-HT release from cortical slices (Nakazi et al., 2000), as well as inhibit serotonin reuptake into rat neocortical synaptosomes (Steffens and Feuerstein, 2004), and in vivo the cannabinoid $CB_1$ receptor antagonist SR141716 stimulates 5-HT release (Tzavara et al., 2003). Furthermore, a diversity of cannabinoid ligands has been shown to reduce the expression of DOI-induced wet dog shakes in rats (Cheer et al., 1999) and head twitches in mice (Darmani, 2001). Recent evidence suggests that, in addition to exogenous cannabinoid ligands, endocannabinoids themselves may suppress 5-$HT_{2A}$ receptor activity. For example, administration of the endocannabinoid anandamide has been shown to reduce the DOI-induced head twitch response in mice; however, this effect was found to be mediated by the downstream metabolites of anandamide and not through $CB_1$ receptor activation (Egashira et al., 2004). Although it remains to be determined if endocannabinoids reduce DOI-induced wet dog shakes in rats, it seems a reasonable possibility given that high concentrations of anandamide reduce binding of ketanserin to the 5-$HT_{2A}$ receptor (Kimura et al., 1998) and wet dog shakes are a symptom of withdrawal from exogenous anandamide (Costa et al., 2000). This suggests that endocannabinoids may act to regulate 5-$HT_{2A}$ receptor functioning. Interestingly, administration of the cannabinoid $CB_1$ receptor antagonist SR141716 also appears to induce the expression of head twitches in mice (Darmani and Pandya, 2000), suggesting that endocannabinoids may actually regulate 5-$HT_{2A}$ receptor functioning in a tonic fashion.

Previous evidence has suggested that the kainate receptors of the glutamatergic system may interact with 5-$HT_{2A}$ receptors. For example, systemic administration of kainic acid induces wet dog shakes (Molla-Hosseini et al., 1985) and direct administration of kainic acid to the dorsal raphe nuclei increases the release of 5-HT (Graeff et al., 1996). Furthermore, the ability of 5-$HT_{2A}$ receptors to elicit a c-fos response in the cortex appears to be contingent upon the 5-$HT_{2A}$ receptor-mediated activation of glutamatergic neurons projecting from the thalamus to the cortex and synapsing onto α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)/kainate receptors (Scruggs et al., 2000). This suggests that AMPA/kainate receptors may play a role in the behavioral effects elicited by 5-$HT_{2A}$ receptor stimulation.

The ability of SR141716 to increase the expression of head twitches in mice is sensitive to both 5-$HT_{2A}$ receptor antagonists as well as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate antagonists (Darmani and Pandya, 2000), suggesting that these three systems may interact functionally. The aim of the present study is to further investigate the influence of the glutamatergic and endocannabinoid systems on 5-$HT_{2A}$ receptor activity in the rat.

## 2. Methods

### 2.1. Subjects

Male Long–Evans rats that were 10 weeks of age and weighed between 300 and 350 g were used in this study. All subjects were housed in groups of three in triple mesh wire cages in a colony room that had a maintained temperature of 21 ± 1 °C and a 12 h:12 h light dark cycle (lights on at 0900 h). All rats had ad libitum access to tap water and Purina Rat Chow, and were handled four times a week prior to testing. All experimental testing on animals was in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the Animal Care Committee of the University of British Columbia.

### 2.2. Drugs

AM404 (*N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide), AM251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) and DNQX (6,7-dinitroquinoxaline-2,3-dione) were obtained from Tocris-Cookson (Bristol, UK). DOI ((±)-2,5-dimethoxy-4-iodoamphetamine) and ketanserin tartrate were obtained from Sigma-Aldrich, Canada. AM404 and AM251 were dissolved in a 1:1:18 solution of dimethyl sulfoxide (DMSO)/Tween 80/0.9% saline. AM404 and AM251 were injected at doses of 1 mg/kg, 5 mg/kg and 10 mg/kg. These doses were based on previous studies examining the effects of these pharmacological agents on behavioral responses and neurochemical responses (Giuffrida et al., 2000; Lastres-Becker et al., 2002; Chen et al., 2004). DNQX was dissolved in DMSO and diluted to 1:1 with saline and administered at doses of 1, 10 and 30 mg/kg based on previous behavioral research (Pierce et al., 1997). DOI and ketanserin were dissolved in 0.9% saline and both were administered at a dose of 1 mg/kg. Injections were given intraperitoneally at a concentration of 1 ml/kg using 26 gauge 0.5 in. stainless steel needles.

### 2.3. Procedure

Animals were taken from their home cages and given an injection of either AM404, AM251, DNQX, ketanserin or vehicle. Thirty minutes following the first injection, subjects were given an injection of DOI and immediately placed in a Plexiglas testing chamber (dimensions 30 cm × 30 cm × 45 cm) layered with contact bedding. For the following 30 min, all animals were monitored and scored by trained observers on the expression of wet dog shakes. All groups had six to seven animals per condition.

### 2.4. Statistics

Total number of wet dog shakes for the 30 min scoring period for each subject was summed, and all behavioral data

were analyzed using a one-way analysis of variance (ANOVA), except for the AM251 data which were analyzed against DOI with a univariate ANOVA to examine for main effects of both drugs. Post hoc analysis was performed using a Tukey's test, and all significance levels were set at a $P$ value of 0.05.

## 3. Results

In line with previous research, 1 mg/kg of DOI elicited a robust increase in wet dog shakes (Gorzalka and Hanson, 1998; Cheer et al., 1999). As expected, pretreatment with ketanserin attenuated the induction of wet dog shakes [$F(2,27)=16.2$, $P<0.01$] such that this group was indistinguishable from the control group. Data regarding the reversal of DOI-induced wet dog shakes by ketanserin can be seen in Fig. 1.

Pretreatment with the endocannabinoid uptake inhibitor AM404 also led to a significant reduction in the expression of DOI-induced wet dog shakes [$F(4,32)=8.4$, $P<0.01$]. Post hoc analysis revealed that this reduction was not significant at the 1 mg/kg or 5 mg/kg doses of AM404 (both $P$'s$>0.05$), but was significant at the 10 mg/kg dose of AM404 ($P<0.05$). Data for the effect of AM404 on wet dog shakes can be viewed in Fig. 2A.

Univariate analysis for AM251 and DOI illustrated that there was a significant interaction between DOI and AM251 [$F(3,43)=3.0$, $P<0.05$], a significant main effect of DOI [$F(1,43)=3277.4$, $P<0.01$], but no significant main effect of AM251 [$F(3,43)=2.3$, $P>0.05$]. Post hoc analysis showed that DOI alone elevated wet dog shakes ($P<0.01$) and that this effect was significantly potentiated by pretreatment with 10 mg/kg AM251 ($P<0.05$), but not by pretreatment with 1 mg/kg or 5 mg/kg of AM251 (both $P$'s$>0.05$). Data for the effects of AM251 on DOI-induced wet dog shakes can be seen in Fig. 2B.

Pretreatment with varying doses of DNQX led to a significant reduction in wet dog shaking frequency [$F(4,29)=15.0$, $P<0.01$]. Post hoc analysis revealed that all three doses of DNQX significantly suppressed DOI-induced wet dog shakes (all $P$'s$<0.01$). The data for the effects of DNQX pretreatment on DOI-induced wet dog shakes can be seen in Fig. 3.

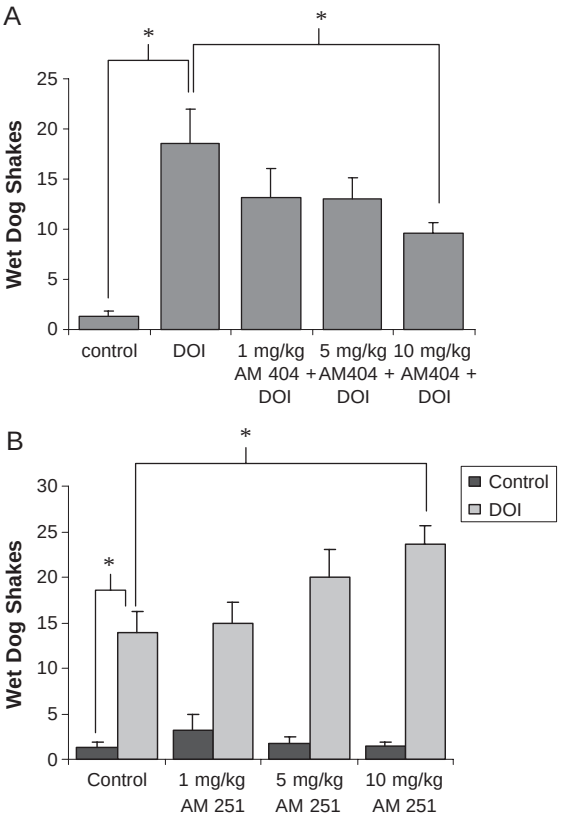


Fig. 2. (A) The effect of DOI (1 mg/kg) administration on wet dog shakes and its attenuation by AM404 (1, 5 and 10 mg/kg). Data are presented as mean wet dog shake frequency±S.E.M. All differences between groups that were significant at $P<0.05$ are denoted by *. (B) The effect of concurrent administration of DOI (1 mg/kg) and AM251 (1, 5 and 10 mg/kg) on wet dog shakes. Data are presented as mean wet dog shake frequency±S.E.M. All differences between groups that were significant at $P<0.05$ are denoted by *.

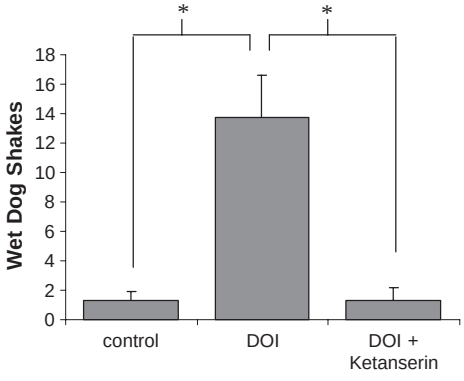


Fig. 1. The effect of DOI (1 mg/kg) administration on wet dog shakes and its reversal by ketanserin (1 mg/kg). Data are presented as mean wet dog shake frequency±S.E.M. All differences between groups that were significant at $P<0.05$ are denoted by *.

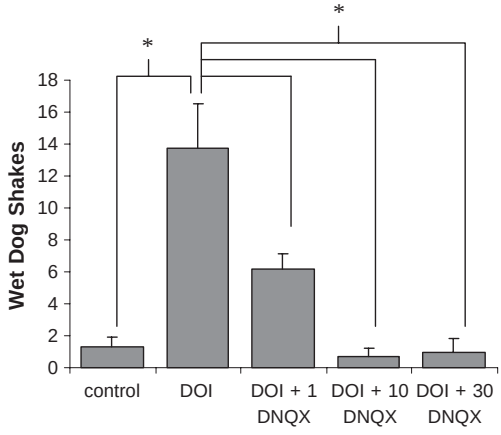


Fig. 3. The effect of DOI (1 mg/kg) administration on wet dog shakes and its attenuation by DNQX (1, 10 and 30 mg/kg). Data are presented as mean wet dog shake frequency±S.E.M. All differences between groups that were significant at $P<0.05$ are denoted by *.

## 4. Discussion

This is the first published research to demonstrate that endocannabinoids have the ability to modulate 5-$HT_{2A}$ receptor-mediated behavioral stereotypies in the rat. Specifically, this experiment demonstrated that, by preventing endocannabinoid uptake, the expression of 5-$HT_{2A}$ receptor-mediated wet dog shakes was reduced. Additionally, blockade of the $CB_1$ receptor resulted in an enhancement of DOI-induced wet dog shakes, suggesting a bidirectional regulation of this phenomenon. Furthermore, it was also shown in this study that blockade of the glutamatergic AMPA/kainate receptors also attenuated DOI-induced wet dog shakes.

This experiment replicated previous research demonstrating that administration of the 5-$HT_{2A/2C}$ receptor agonist DOI robustly induces the expression of wet dog shakes. To ensure that this effect was mediated by 5-$HT_{2A/2C}$ receptors, we demonstrated that the behavior was completely suppressed by pretreatment with the 5-$HT_{2A/2C}$ receptor antagonist ketanserin. We have previously demonstrated that ketanserin alone has no effect on wet dog shakes except when 5-$HT_{2A}$ receptors are activated (Gorzalka et al., 2001). Previous research revealed that exogenous cannabinoid ligands can suppress DOI-induced behavioral stereotypies (Cheer et al., 1999; Darmani, 2001). Subsequently, this effect was also found following exogenous administration of the endocannabinoid anandamide to mice (Egashira et al., 2004), which lead to the hypothesis that the endocannabinoid system itself may play a role in modulation of 5-$HT_{2A}$ receptor activity. The present data support the hypothesis that endocannabinoids have the ability to reduce 5-$HT_{2A}$ receptor activity as demonstrated by the ability of the endocannabinoid uptake inhibitor AM404 to reduce the expression of DOI-induced wet dog shakes. However, due to the ability of $CB_1$ receptor antagonists to stimulate 5-HT release (Tzavara et al., 2003) and cannabinoidergic ligands to suppress it (Nakazi et al., 2000), the possibility also exists that these agents could be altering the regional levels of synaptic 5-HT and not actually directly affecting the 5-$HT_{2A}$ receptor. Furthermore, while the bidirectional response of these effects suggests that these effects are mediated by either enhancement or blockade of the endocannabinoid system, due to the pharmacological non-specificity of AM404, it cannot be ruled out that these effects may in part be mediated by actions at the vanilloid receptor (Lastres-Becker et al., 2003).

However, there were discrepancies between this research and previous studies; specifically AM251 alone did not induce wet dog shakes. This finding differs from previous data demonstrating that another $CB_1$ receptor antagonist, SR141716, does increase head twitches in mice when administered alone (Darmani and Pandya, 2000), but was consistent with data in rats where SR141716 did not induce wet dog shake expression (Cheer et al., 1999). These differences in findings are likely due to either the species difference or age of the subjects. However, it is unlikely that this effect is a species difference as some studies have shown that rats exhibit wet dog shakes following acute administration of SR141716A (Rubino et al., 2000). As put forth by Darmani and Pandya (2000), this difference is likely due to an age difference, as studies demonstrating that $CB_1$ receptor antagonists have the ability to stimulate wet dog shake expression are often done using younger animals. The animals used in this study were adults, suggesting that possibly the coupling of the endocannabinoid system with the 5-$HT_{2A}$ receptor may be more integrated during early development, as induction of wet dog shakes by administration of SR141716 alone suggests that there is a basal endocannabinoid tone that persistently suppresses 5-$HT_{2A}$ receptor activity. Perhaps there is a transient association between these systems during development that tapers with age; however, ontogenetic studies will be required to fully characterize the time course of this interaction.

The current findings do not suggest that endocannabinoids consistently regulate 5-$HT_{2A}$ receptor activity in the adult, but perhaps do so under conditions of elevated activity. This possibility is supported by the biochemical effects of 5-$HT_{2A}$ receptor stimulation. 5-$HT_{2A}$ receptor stimulation leads to activation of phospholipase C, which in turn leads to hydrolysis of inositol-containing phospholipids resulting in the production of diacylglycerol (Berg et al., 1998). A sn-1 specific diacylglycerol lipase can then convert diacylglycerol into 2-arachidonylglycerol, the most abundant endocannabinoid in the brain (Bisogno et al., 2004). This suggests that, during periods of elevated 5-$HT_{2A}$ receptor activity, such as that following administration of the potent agonist DOI, endocannabinoid levels in the brain would become elevated. This increase in endocannabinoid content could then act as a buffer to regulate 5-$HT_{2A}$ receptor activity through a mechanism yet to be determined. Previous in vitro work has shown that high levels of anandamide, another endocannabinoid, can reduce the binding of ketanserin to the 5-$HT_{2A}$ receptor (Kimura et al., 1998), suggesting that endocannabinoids in high concentrations may directly interfere with serotonin binding to the 5-$HT_{2A}$ receptor. However, the concentrations of anandamide used in that study were extremely high and may not predict physiological events in vivo.

Given the role AMPA/kainate receptors have in the activation of the cortex by DOI (Scruggs et al., 2000), we examined how AMPA/kainate antagonism influenced wet dog shake expression. It was found that pretreatment with a variety of doses of the AMPA/kainate antagonist DNQX resulted in a significant suppression of wet dog shakes. This finding strongly supports those of Scruggs et al. (2000), who showed that AMPA/kainate receptor antagonism could completely block the c-Fos induction elicited by DOI in the

cortex. This also supports the hypothesis put forth by Scruggs et al. (2000) that AMPA/kainate receptors are integral to the effects of DOI and suggests that the induction of wet dog shakes may be due in part to activation of specific cortical sites.

The interaction between the glutamatergic system and the 5-HT system may prove to be the locus of action for endocannabinoids. While $CB_1$ receptors have not been immunohistochemically detected on glutamatergic fibers, elegant pharmacological work has demonstrated that a yet uncharacterized cannabinoid receptor does exist on glutamate terminals (Hajos and Freund, 2002). This idea is supported by electrophysiological findings that cannabinoid ligands possess the ability to suppress glutamatergic activity (Shen et al., 1996). More interesting is the fact that the endocannabinoid system appears to be activated by administration of kainic acid, and acts as a regulatory buffer to limit the excitotoxicity induced by intense kainate receptor activation (Marsicano et al., 2003). This suggests that, instead of acting directly on the $5\text{-}HT_{2A}$ receptor as previously suggested, endocannabinoids may actually act upstream at the glutamatergic junction of the signal cascade initiated by activation of the $5\text{-}HT_{2A}$ receptor. While this idea is supported by findings from Darmani and Pandya (2000) who demonstrated that head twitches induced in mice by the $CB_1$ receptor antagonist SR141716A were attenuated by pretreatment with both a $5\text{-}HT_{2A/2C}$ receptor antagonist and an AMPA/kainate receptor antagonist, the hypothesis requires further testing.

The current findings may help to develop novel pharmacotherapeutic agents for treatment of various mood and behavioral disorders which involve the $5\text{-}HT_{2A}$ receptor. For example, stimulation of $5\text{-}HT_{2A}$ receptors results in a reduction in feeding behavior (Kitchener and Dourish, 1994; Raghavendra and Kulkarni, 2000), a finding that is interesting in light of data demonstrating that individuals suffering from anorexia have increased binding of platelet $5\text{-}HT_{2A}$ receptors (Spigset et al., 1999). Both AMPA/kainate antagonists and cannabinoidergic agents have been shown to stimulate feeding behavior (Hettes et al., 2003; Berry and Mechoulam, 2002), suggesting that understanding the relationship between these systems and the $5\text{-}HT_{2A}$ receptor may contribute to knowledge of the etiology and development of treatment for eating disorders.

These findings confirm and extend previous findings in the area examining the interaction between the serotonergic, glutamatergic and endocannabinoid systems. Moreover, they suggest that $5\text{-}HT_{2A}$ receptor activity is negatively modulated by endocannabinoids and positively regulated by AMPA/kainate receptor activation.

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