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## Effects of short- and long-term aripiprazole treatment on Group I mGluRs in the nucleus accumbens: Comparison with haloperidol

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### Abstract

The D2 receptor partial agonist, aripiprazole, has shown increased therapeutic efficacy for schizophrenia, autism and Tourette's syndrome compared to traditional antipsychotics such as the D2 receptor antagonist, haloperidol. Recent evidence suggests this superior profile may be associated with downstream effects on glutamatergic synapses. Group 1 metabotropic glutamate receptors (mGluRs) and their endogenous modulators, Norbin and Homer1, are regulated by D2 receptor activity, particularly within the nucleus accumbens (NAc), a target region of aripiprazole and haloperidol. This study sought to evaluate the effects of aripiprazole on Group 1 mGluRs, Norbin and Homer1 in the NAc, in comparison to haloperidol. Sprague-Dawley rats were orally administered daily doses of aripiprazole (2.25 mg/kg), haloperidol (0.3 mg/kg) or vehicle for 1 or 10-weeks. Immunoblot analyses revealed Group 1 mGluR protein levels were not altered following 1-week and 10-week aripiprazole or haloperidol treatment, compared to vehicle treated rodents. However, 1-week aripiprazole and haloperidol treatment significantly elevated Homer1a and Norbin protein expression, respectively. After 10 weeks of treatment, aripiprazole, but not haloperidol, significantly increased Norbin expression. These findings indicate the antipsychotics, aripiprazole and haloperidol, exert differential temporal effects on Norbin and Homer1 expression that may have consequences on synaptic glutamatergic transmission underlying their therapeutic profile.

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## Effects of short- and long-term aripiprazole treatment on Group I mGluRs in the nucleus accumbens: comparison with haloperidol

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### Abstract:

The D2 receptor partial agonist, aripiprazole, has shown increased therapeutic efficacy for schizophrenia, autism and Tourette's syndrome compared to traditional antipsychotics such as the D2 receptor antagonist, haloperidol. Recent evidence suggests this superior profile may be associated with downstream effects on the glutamatergic synapses. Group 1 metabotropic glutamate receptors (mGluRs) and their endogenous modulators, Norbin and Homer1, are regulated by D2 receptor activity, particularly within the nucleus accumbens (NAc), a target region of aripiprazole and haloperidol. This study sought to evaluate the effects of aripiprazole on Group 1 mGluRs, Norbin and Homer1 in the NAc, in comparison to, haloperidol. Sprague-Dawley rats were orally administered aripiprazole (0.75 mg/kg), haloperidol (0.1 mg/kg) or vehicle, 3 times/day for 1- or 10-weeks. Immunoblot analyses revealed Group 1 mGluR proteins levels were not altered following 1-week and 10-week aripiprazole or haloperidol treatment, compared to vehicle treated rodents. However, 1-week aripiprazole and haloperidol treatment significantly elevated Homer1a and Norbin protein expression, respectively. After 10-weeks of treatment, aripiprazole, but not haloperidol, significantly increased Norbin expression. These findings indicate the antipsychotics, aripiprazole and haloperidol exert differential temporal effects on Norbin and Homer1 expression that may have consequences on synaptic glutamatergic transmission underlying their therapeutic profile.

**Keywords:** mGluR1, mGluR5, Norbin, Homer1a, Homer1b/c, ventral striatum, antipsychotic

**Highlights:**

Aripiprazole and haloperidol have temporal-dependent effects on Group 1 metabotropic glutamate receptor endogenous regulators, Norbin and Homer1, in the nucleus accumbens.

1-week aripiprazole and haloperidol treatment increased Homer1a and Norbin expression, respectively, in the nucleus accumbens.

10-week aripiprazole, but not haloperidol treatment, increased Norbin expression in the nucleus accumbens.

Group 1 metabotropic glutamate receptor protein levels are not altered in the nucleus accumbens following 1- and 10-week aripiprazole treatment.

## **1. Introduction**

Aripiprazole is a novel atypical antipsychotic drug originally developed for the treatment of schizophrenia. However, more recently, aripiprazole is employed to treat symptoms of several disorders including bipolar disorder, autism spectrum disorder, Tourette's syndrome, mood disorders and more recently has been identified as a possible treatment option for substance abuse (for reviews see (García-Amador et al., 2006; Greenaway and Elbe, 2009; Moreira and Dalley, 2015; Yang et al., 2015). Aripiprazole has a unique pharmacological profile, acting primarily as a dopamine D2 receptor partial agonist (Shapiro et al., 2003). In contrast, customary antipsychotics such as haloperidol, exhibit dopamine D2 receptor antagonist properties, a characteristic traditionally thought to be vital for antipsychotic efficacy (Kapur and Mamo, 2003). The nucleus accumbens (NAc) contains an abundance of D2 receptors and is believed to be central in the pathology of many of the above disorders, in addition to mediating the effects of antipsychotics (Deutch et al., 1992; Deutch and Cameron, 1992). Haloperidol and aripiprazole target the D2 receptor, albeit in different ways; understanding their neurochemical commonalities and differences in the NAc may provide insight into their potential therapeutic mechanism for not only schizophrenia, but other neuropsychiatric disorders.

While aripiprazole, haloperidol and many other antipsychotic drugs have direct effects on the dopaminergic system, increasing evidence suggests antipsychotics modulate the glutamatergic system, particularly within the striatum. Several studies demonstrate antipsychotics, including haloperidol and aripiprazole, can alter the ligand binding, mRNA and protein expression of glutamatergic ionotropic receptors, in particular AMPA and NMDA receptors, within the NAc (Fitzgerald et al., 1995; Healy and Meador-Woodruff, 1997; Pan et al., 2016a; Schmitt et al., 2003; Spurney et al., 1999). AMPA and NMDA receptor activity and cell surface expression are modulated by the Group 1 metabotropic glutamate receptors (mGluR1 and mGluR5) (Awad et al., 2000; Kammermeier, 2008). Within the striatum, increasing evidence supports a physical and functional relationship between Group 1 mGluRs and the D2 receptor (Diaz-Cabiale et al., 2002). Antagonism of the D2 receptor with eticlopride was shown to increase the abundance of synaptic mGluR5 and tyrosine phosphorylation of mGluR5 in cultured striatal neurons (Mao and Wang, 2016). Furthermore, recent evidence demonstrated chronic, but not acute, treatment with the D2 receptor agonist, quinpirole, increased in vivo mGluR5 binding in the rat NAc, indicating time-dependent effects of D2 receptor activity on Group 1 mGluRs (Servaes et al., 2017).

Group 1 mGluRs rely on interactions with various endogenous proteins, including Norbin and Homer1, which are localised within excitatory glutamatergic postsynaptic terminals and interact directly with the C-terminus of these mGluRs. Norbin and Homer1 both regulate mGluR5 cell surface expression and downstream signalling and play a large role in synaptic remodelling (Brakeman et al., 1997; Wang et al., 2009). Homer1 is a group of scaffolding proteins responsible for the configuration and signal transduction of proteins located within excitatory synapses, acting as a multi-valent protein for Group 1 mGluRs. The long isoforms of Homer1, Homer1b and Homer1c, are constitutively expressed and enable coupling of Group 1 mGluRs to IP<sub>3</sub> receptors and subsequently facilitate intracellular Ca<sup>2+</sup> release (Mao et al., 2005). However, the short form, Homer1a, is an immediate early gene, which is upregulated in response to synaptic activity (Brakeman et al., 1997). Homer1a acts in a dominant negative manner, by competitively binding to Group 1 mGluRs and favouring IP<sub>3</sub> independent signalling pathways. Consequently, shifts in the ratio of short:long forms of Homer1 alter Group 1

mGluR downstream signalling (Kaja et al., 2013; Kammermeier, 2008) and highlight the biological significance in not only measuring individual levels of Homer1 isoforms, but the respective ratio of these proteins. Reports from De Bartolomesi and colleagues have repeatedly shown that haloperidol treatment alters Homer1a and Homer1b/c mRNA in the NAc, leaving a question as to the effect on Homer1 protein expression in this region (Ambesi-Impimbato et al., 2007; de Bartolomeis et al., 2013, 2002, Iasevoli et al., 2011, 2010b, 2010a; Tomasetti et al., 2007).

Collectively, the evidence suggests D2 receptor activity can modify the activity, expression and localisation of Group 1 mGluRs and their endogenous regulators, such as Homer1. The NAc contains an abundance of D2 receptors and is a point of convergence for the dopaminergic and glutamatergic system. Furthermore, the NAc is a brain region related to the pathology and treatment of many disorders prescribed aripiprazole or haloperidol and therefore these compounds may alter the aforementioned glutamatergic proteins, which may underlie their therapeutic efficacy. The aim of this study was to examine the effect of short- and long-term antipsychotic treatment with the D2 partial agonist, aripiprazole, and the D2 antagonist, haloperidol, on the protein expression of Group 1 mGluRs, Homer1 and Norbin in the NAc and provide a deeper insight into their clinical pharmacological profile.

## **2. Methods**

### **2.1 Animals housing:**

Male Sprague Dawley rats (8 weeks old) were acquired from the Animal Resource Centre (Perth, Australia). Rats were housed individually under controlled environmental conditions (temperature 22°C, light cycle from 07:00 to 19:00 h) with *ad libitum* access to water and standard laboratory chow. All experimental procedures were approved by the Animal Ethics Committee (AE11/02), University of Wollongong, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

### **2.2 Short and long term treatment paradigm**

Animal treatments and the drug delivery method have been previously described in Pan et al., (Pan et al., 2016c, 2016a). In short, rats were trained for self-administration of cookie dough pellets (containing sucrose 30.9%, corn starch 30.9%, casein 15.5%, minerals 8.4%, fibre 6.4%, gelatine 6.3% and vitamins 1.6%) a week prior to drug treatment. At 10 weeks of age, rats were randomly assigned into one of the following three groups ( $n=6/\text{group}$ ): haloperidol (0.3 mg/kg/day), Sigma, Castle Hill, Australia); aripiprazole (2.25 mg/kg/day Otsuka, Tokyo, Japan); or vehicle. The daily dosage was divided into three equal amounts and rats were fed cookie dough pellets (containing drug treatments or vehicle) three times a day (0700-, 15:00- and 2300 hr) for either 1- or 10-weeks. Rats were observed following pellet delivery to confirm the consumption of each pellet. Treatment dosages were derived from recommended clinical dosages based on body surface area, in accordance with current FDA guidelines (FDA, 2005.; Reagan-Shaw et al., 2008). Daily dosages of 0.3 mg/kg/day haloperidol and 2.25 mg/kg/day aripiprazole are equivalent to ~3.0 and ~22.5 mg in humans (based on 60 kg body weight), respectively, with these transferred daily dosages within the recommended clinical dosages (Gardner et al., 2010; Mace and Taylor, 2009). Furthermore, the dosages of haloperidol and aripiprazole have previously shown to

induce 70 and 90% D2 receptor occupancy, respectively (Kapur et al., 2003; Naiker et al., 2006; Natesan et al., 2005). Moreover, the dosages used in the present study have shown to display physiological and behavioural effects in rodents, with the absence of extra pyramidal side-effects (Assié et al., 2006; De Santis et al., 2014; Han et al., 2009; Kesby et al., 2006). Rats were euthanised by CO<sub>2</sub> asphyxiation 12 hours following the last treatment. Brains were immediately collected and snap frozen in liquid nitrogen.

### 2.3 Microdissection and tissue processing

The NAc (core and shell) was collected using a brain microdissection puncture technique, as previously described (De Santis et al., 2014). In brief, 500 µm thick coronal sections of the rat brain were cut at -14°C and mounted on glass slides. The NAc was identified at approximately Bregma +1.00 mm using a standard rat brain atlas (Paxinos and Watson, 2007); the NAc was identified, punctured (left/right hemispheres combined), and stored at -80°C. Tissue was homogenised in NP-40 cell lysis buffer (Invitrogen, Australia) containing Protease Inhibitor Cocktail (P8340; Sigma, Australia) and Phosphatase Inhibitor Cocktail 2 (Sigma, Australia). Protein concentrations were determined using the DC Protein Assay (Bio-Rad, #500-0111).

### 2.4 Western blot analyses

Independent immunoblot analyses were performed on short- and long-term treated tissue, as previously described (Lum et al., 2016). In brief, samples containing 5 µg of protein were resolved in 4-20% TGX precast gels (Bio-Rad, Australia), and subsequently transferred to a PVDF membrane (Bio-Rad). This loading concentration was determined to be in the linear range of a standard curve (Lum et al., 2017). The membranes were incubated in the primary polyclonal antibodies at the following concentrations: anti-mGluR1α (1:15 000; DH510, Cell Signalling), anti-mGluR5 (1:5000; ab29170, Abcam), anti-Norbin (1:7500; Ab130507; Abcam), anti-Homer1a (1:5000; sc-8922, Santa Cruz) and anti-Homer1b/c (1:10 000; Ab97593; Abcam). Membranes were subsequently incubated with horseradish peroxidase conjugated secondary antibodies. Bands were visualised using chemiluminescence detection reagents (GE Healthcare, Australia) and membranes exposed to Hyperfilm (GE Healthcare, Australia). Films were scanned using a GS-800 scanner (Bio-Rad) and densitometry values were quantified. Relative densitometry values for each protein were normalised to their respective β-actin levels and an internal control value (containing equal amounts of all samples), to account for protein loading and gel-to-gel variability, respectively. Each sample was run in duplicate.

### 2.5 Statistical analysis

One-way Analyses of Variance (ANOVA) were used to identify any effects of antipsychotic treatment on protein density for each treatment duration, followed by post-hoc Bonferroni corrections. Where data showed unequal variances, Kruskal-Wallis tests were used to determine significant effects of antipsychotic treatment. All data were analysed using SPSS Statistics V21.0. Significance was set at an alpha level of  $p < 0.05$ .

## **3. Results**

To examine the effects of antipsychotics on Group 1 mGluR, Norbin, Homer1a and Homer1b/c protein levels immunoblots were performed on rat NAc tissue following 1- and 10-week treatment with either haloperidol, aripiprazole or vehicle. Group 1 mGluRs are predominantly expressed in their functional homodimeric state in

the adult brain (Lum et al., 2016; Moustaine et al., 2012). Therefore, mGluR1 and mGluR5 dimer bands, respectively, were quantified in the present study. mGluR1 and mGluR5 dimers, presented as two bands at their expected dimeric molecular weight (270-280 kDa), respectively (Figure 1a and 2a). We and others have previously reported this pattern of bands for Group 1 mGluRs (Kirschstein et al., 2007; Lee et al., 2015; Lum et al., 2016), including with the use of alternate mGluR1 and mGluR5 antibodies and specificity has been confirmed using respective knockout mice (Ayala et al., 2012; Lee et al., 2015). Norbin, Homer1a and Homer1b/c were each observed as a single band at their expected molecular weight (74-, 21- and 48 kDa, respectively; Figure 1a and 2a) (Lum et al., 2016; Matosin et al., 2016).

### 3.1 Short-term aripiprazole and haloperidol treatment differentially increase Norbin and Homer1a protein levels in the NAc

One-week of antipsychotic drug treatment did not significantly affect mGluR1 ( $F_{2,15}=0.808$ ;  $p=0.467$ ) or mGluR5 ( $F_{2,15}=0.876$ ;  $p=0.440$ ) protein levels in the NAc (Figure 1b and c). However, protein expression of the Group 1 endogenous regulator, Norbin, was significantly altered after 1-week of antipsychotic treatment with haloperidol, compared to vehicle treated rats (41%;  $p=0.036$ ) (Figure 1d). Although visual appearance of the means showed a similar trend for aripiprazole treatment compared to vehicle treated rats, this did not reach statistical significance ( $p=0.270$ ). Conversely, 1-week aripiprazole treatment significantly increased Homer1a protein expression, compared to vehicle (87%;  $p<0.001$ ) and haloperidol (62%;  $p<0.001$ ) treated rats (Figure 1e). Whilst, Kruskal-Wallis tests revealed Homer1b/c protein expression was unaffected by 1-week antipsychotic drug treatment ( $X^2:3.559$ ;  $p=0.169$ ) (Figure 1f). Consequently, aripiprazole treated rats showed a significant increase of the ratio of Homer1a:Homer1b compared to vehicle treated rats (156%;  $p=0.006$ ) (Figure 1g).

### 3.2 Long-term aripiprazole treatment increases Norbin protein levels in the NAc

Non-parametric tests showed mGluR1 and mGluR5 dimer protein levels within the NAc were not significantly affected by 10-week antipsychotic drug treatment ( $X^2=1.014$ ;  $p=0.602$  and  $F_{2,17}=1.329$ ;  $p=0.294$ , respectively)(Figure 2b and c). However, analysis of Norbin expression following 10-week antipsychotic treatment revealed aripiprazole treated rodents exhibited increased Norbin protein expression compared to both vehicle (25%;  $p=0.012$ ) and haloperidol treated rats (18%;  $p=0.052$ ), with haloperidol treated rats showing no significant difference in Norbin levels compared to vehicle controls ( $p=0.730$ ) (Figure 2d). Homer1 protein levels were not altered following 10-week antipsychotic treatment (Homer1a:  $F_{2,14}=2.894$ ;  $p=0.094$ ; Homer1b/c:  $F_{2,16}=0.894$ ;  $p=0.431$ ; Homer1a:Homer1b/c:  $F_{2,14}=1.943$ ;  $p=0.186$ ) (Figure 2e-g).

## 4. Discussion:

Haloperidol and aripiprazole primarily target the striatal dopaminergic system and have little affinity for glutamatergic receptors; however, evidence suggests antipsychotic treatment can indirectly modulate the glutamatergic system. Here we examined the protein expression of Group 1 mGluRs, Norbin, Homer1a and Homer1b/c following 1-week or 10-week treatment with the D2 partial agonist, aripiprazole or the D2 antagonist, haloperidol. Whilst several previous studies suggest that the mRNA expression of these genes is upregulated following antipsychotic treatment, this is the first study to examine these genes at a protein level.



We report whilst Group 1 mGluR expression is unaltered following short- and long-term haloperidol and aripiprazole treatment, protein levels of their endogenous regulators, Homer1 and Norbin are differentially altered in the NAc.

Haloperidol and aripiprazole act via D2 receptors, particularly within the NAc, however, repeated studies demonstrate their capacity to alter glutamatergic tone within this region (Fitzgerald et al., 1995; Healy and Meador-Woodruff, 1997; Schmitt et al., 2003; Yamamoto and Cooperman, 1994). Indeed, we previously reported increased expression of the NR1 NMDA receptor subunit in the NAc following 10-week haloperidol and aripiprazole treatment in the same cohort of chronic treated rodents as the present study (Pan et al., 2016b). However, in the present study, we did not observe any changes to either mGluR1 or mGluR5 protein levels following short- or long-term haloperidol or aripiprazole treatment. These results are in line with our previous findings, where we found no change in [<sup>3</sup>H]MPEP binding within the striatum of rodents chronically treated with either haloperidol or olanzapine (Matosin et al., 2013). In addition, this is largely consistent with mRNA transcript expression in the NAc (Iasevoli et al., 2010b) and striatum (Tascedda et al., 2001) following chronic (21 day) treatment with haloperidol, clozapine, olanzapine or sertindole. Despite previous studies indicating antipsychotic treatment can alter glutamatergic tone within the striatum and specifically the NAc, collectively, the present and previous studies suggests this does not extend to the binding, mRNA and protein expression of Group 1 mGluRs.

Whilst the expression of Group 1 mGluRs was unperturbed following antipsychotic treatment in the present study, we cannot exclude that synaptic localisation of mGluR1 or mGluR5 was not altered. Dopaminergic activity, specifically through the D2 receptor, has shown to modulate the localisation of mGluR5. D2 receptor antagonism with eticlopride has previously shown to increase the expression of mGluR5 specifically at the synaptic membrane (Mao and Wang, 2016). The Group 1 mGluR endogenous regulator, Norbin, has shown to increase mGluR5 cell surface expression (Wang et al., 2009). Furthermore, neuronal cells overexpressing Norbin have shown to increase length of mGluR5 induced Ca<sup>2+</sup> oscillations, IP<sub>3</sub> accumulations and ERK1/2 phosphorylation (Wang et al., 2009). We report Norbin expression was increased by both haloperidol and aripiprazole, however in a time-dependent manner. Whilst it is unclear why we observe this temporal response, changes to Norbin expression following antipsychotic treatment may indicate synaptic mGluR5 expression and signalling is consequently altered. Furthermore, increased Norbin expression is associated with increases in neurite length (Shinozaki et al., 1997, 1999), whilst Norbin KO mice show synaptic deficits (Wang et al., 2009). Aripiprazole was shown to increase neurite length in cultured cells, which is blocked with a specific IP<sub>3</sub> receptor inhibitor and BAPTA-AM, a Ca<sup>2+</sup> chelating agent (Ishima et al., 2012). This indicates Norbin-mediated processes, such as IP<sub>3</sub> receptor activation and subsequent intracellular Ca<sup>2+</sup> release is vital for aripiprazole-induced neurite growth. In contrast to our findings, de Bartolomeis et al., (2013) reported Norbin mRNA expression as unchanged within the NAc, however this was following a single dose of haloperidol or atypical antipsychotic, amisulpride. Furthermore, we have previously reported no change to cortical and hippocampal Norbin expression following 8-, 15- and 35-day antipsychotic treatment (Matosin et al., 2015, 2016), suggesting these effects may be brain region specific. Further studies will need to confirm if the observed changes in Norbin expression in the NAc influences its physical/functional interaction with Group 1 mGluRs, is associated

with neurite morphology and if similar effects are observed for other endogenous regulators of Group 1 mGluRs.

Similar to Norbin, Homer1 isoforms have been shown to regulate Group 1 mGluR cell surface expression and downstream signalling within striatal neurons (Mao et al., 2005). Whilst we did not observe any significant changes to Homer1a following haloperidol treatment, we did observe an increase of Homer1a protein levels within the NAc following 1-week aripiprazole treatment, which subsequently caused a significant shift towards expression of the short form of Homer1. This did not extend to the 10-week aripiprazole treatment, suggesting aripiprazole-induced Homer1a desensitisation may occur. In line with this, a previous study reported rodents acutely treated with aripiprazole showed an increase in Homer1a mRNA, however chronic aripiprazole treatment (21 days) showed no effect on Homer1a mRNA levels (Tomasetti et al., 2007). Induction of Homer1a expression has previously been described via several mechanisms; D1 receptor activity, NMDA receptor mediated  $\text{Ca}^{2+}$  influx and NMDA-independent-BDNF activation (Sato et al., 2001; Zhang et al., 2007). Although aripiprazole does not possess affinity for the D1R (Shapiro et al., 2003), its ability to competitively bind to postsynaptic D2 receptor, may increase the probability of dopamine to bind/activate the D1R and subsequently stimulate Homer1a activation via D1 receptors. However, we would have expected to observe a similar pattern with haloperidol, therefore it is more likely aripiprazole may induce Homer1a expression through alternative mechanisms. Aripiprazole has been shown to increase BDNF expression in SH-SY5Y cells, a phenomenon not observed following haloperidol treatment (Park et al., 2009), which has been suggested may be due to aripiprazole's agonist activity of the 5-HT<sub>1A</sub> receptor (Shapiro et al., 2003). However, further studies are required to identify direct causes. Furthermore, it will be of interest to identify how aripiprazole-induced increases of Homer1a may influence Group 1 mGluR signalling. Modest increases of endogenous Homer1a levels has been shown to uncouple mGluR5 from IP<sub>3</sub> dependent signalling and their modulation of excitatory postsynaptic currents (Kammermeier, 2008). Aripiprazole induced Homer1a expression may have advantageous implications for its treatment, considering inhibition of mGluR5 has been identified as a possible therapeutic avenue for substance abuse, depression and autism (Aguilar-Valles et al., 2015; Belozertseva et al., 2007; Brodtkin et al., 2002; Popik and Wróbel, 2002).

Although it is likely, the observed differences of short- and long-term treatment are due to the duration of antipsychotic treatment, one aspect that must be considered is the possible effect of ageing, in regards to the differences observed between treatment durations. Whilst all animals commenced treatment at the same age (10 weeks old), following short- and long-term treatment the rats were euthanised and subsequently analysed at 11 and 20 weeks old, respectively. We and others have previously reported that the density and binding of the D2 receptor plateaus following adolescence in rodents (Andersen et al., 1997; du Bois et al., 2008; Tarazi and Baldessarini, 2000). Thus, it is unlikely that expression of the D2 receptor, the primary target of aripiprazole and haloperidol, drastically changed over the duration of the antipsychotic treatment period due to aging. The expression and functionality of proteins investigated in the present study, such as mGluR5 and Homer1a have been reported to slowly decline with age (Domenici et al., 2003; Kaja et al., 2013), in line with age-dependent reductions in striatal synaptic plasticity (Cepeda et al., 1989; Ou et al., 1997). However, these observed changes occur at significantly later time points than the age of rodents and duration of antipsychotic treatment in the

present study, suggesting the effects observed in the current study are indeed due to the length of antipsychotic treatment duration and not ageing.

The present study demonstrates haloperidol and aripiprazole treatment influences the glutamatergic system in the NAc. Haloperidol and aripiprazole exhibit a temporal-dependent increase of two major endogenous regulators of Group 1 mGluRs, Norbin and Homer1a. Whilst we did not observe concurrent changes to the protein expression of mGluR1 and mGluR5, considering the roles of Homer1 and Norbin, these changes could potentially alter Group 1 mGluR cellular localisation, downstream signalling and synaptic plasticity. However, future studies should investigate if these changes alter the physical interaction between these proteins. In conclusion, these findings aid in understanding the molecular adaptations of haloperidol and aripiprazole that may underlie their therapeutic efficacy for many neurological disorders.

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### **Conflicts of Interest**

All authors declare no conflicts of interest.

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#### Figure captions:

**Figure 1:** **a.** Representative immunoblots of dimeric mGluR1, dimeric mGluR5 and endogenous regulators Norbin, Homer1a and Homer1b/c in the nucleus accumbens following 1 week haloperidol (HAL), aripiprazole (ARI) and vehicle (VEH) treatment. Representative  $\beta$ -actin is shown for Homer1a blots only. Graphs illustrate percentage differences of **b.** dimeric mGluR1, **c.** dimeric mGluR5, **d.** Norbin, **e.** Homer1a, **f.** Homer1b/c and **g.** the ratio of Homer1a:Homer1b/c in the nucleus accumbens following 1 week haloperidol, aripiprazole and vehicle treatment. Data are expressed as mean percentage of vehicle treated rodents + SEM. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ .

**Figure 2:** **a.** Representative immunoblots of dimeric mGluR1, dimeric mGluR5 and endogenous regulators Norbin, Homer1a and Homer1b/c in the nucleus accumbens following 10 week haloperidol (HAL), aripiprazole (ARI) and vehicle (VEH) treatment. Representative  $\beta$ -actin is shown for Homer1a blots only. Graphs illustrate percentage differences of **b.** dimeric mGluR1, **c.** dimeric mGluR5, **d.** Norbin, **e.** Homer1a, **f.** Homer1b/c and **g.** the ratio of Homer1a:Homer1b/c in the nucleus accumbens following 10 week haloperidol, aripiprazole and vehicle treatment. Data are expressed as mean percentage of vehicle treated rodents + SEM. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ .





