Haloperidol-induced immediate early genes in striatopallidal neurons requires the converging activation of cAMP/PKA/DARPP-32 and mTOR pathways

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Abstract

Antipsychotics share the common pharmacological feature of antagonizing the dopamine 2 receptor (D2R) which is abundant in the striatum and involved in both the therapeutic and side effects of this drugs class. Pharmacological blockade of striatal D2R, by disinhibiting the D2R-containing medium-size spiny neurons (MSNs), leads to a plethora of molecular, cellular and behavioral adaptations which are central in the action of antipsychotics.

Here, we focused on the cell type-specific (D2R-MSNs) regulation of some striatal immediate early genes (IEGs), such as cFos, Arc and Zif268. Taking advantage of transgenic mouse models, pharmacological approaches and immunofluorescence analyses, we found that haloperidol-induced IEGs in the striatum required the synergistic activation of A2a (adenosine) and NMDA (glutamate) receptors. At the intracellular signaling level, we found that the PKA/DARPP-32 and mTOR pathways synergistically cooperate to control the induction of IEGs by haloperidol.

By confirming and further expanding previous observations, our results provide novel insights into the regulatory mechanisms underlying the molecular/cellular action of antipsychotics in the striatum.

Introduction

The vast majority of antipsychotics share the common pharmacological feature of antagonizing the dopamine (DA) D2 receptors (D2Rs). These receptors are abundant in the striatum where they are found in half of striatal medium-size spiny neurons (MSNs) and in cholinergic interneurons but also on terminals of projecting DA-neurons [1,2].

D2R-MSNs represent the main cell type through which antipsychotics (*i.e.* haloperidol, raclopride, eticlopride, sulpiride) can lead to extrapyramidal and motor side effects such as hypolocomotion, catalepsy, parkinsonism and tardive dyskinesia [3,4]. A large body of evidence indicates that D2R antagonists, by blocking/preventing the DA→D2R→Gi-mediated tonic inhibition of D2R-MSNs, promote the activation/disinhibition of intracellular signaling events through the recruitment of multiple pathways, including the cAMP-dependent protein kinase A (PKA)/dopamine- and cAMP-regulated phosphoprotein (DARPP-32) and the mTOR cascades [5–9], which participate in the regulation of both transcriptional and translational modifications. However, although blockade of the endogenous action of DA onto D2R is necessary to promote the activation of signaling cascades in D2R-MSNs, several reports have also shown that D2R downstream intracellular signaling events (*i.e.* cAMP/PKA pathway) also depend on the dynamic orchestration of several extracellular and intracellular players whose activity may scale the modulation of D2R-containing neurons [7–13], thus reflecting the nature of MSNs as coincidence detectors of different neurochemical stimuli. Within the striatum, this antipsychotics-associated signaling cascade rapidly culminate in the long-term expression of immediate early genes (IEGs), such as cFos, Arc and Zif268 [14].

IEGs represent a heterogenous class of highly responsive genes that can be rapidly and transiently induced by a plethora of physiological, pharmacological and environmental stimuli. The encoded proteins of IEGs cFos, Arc and Zif268 are well-known to contribute to forms of synaptic plasticity [15]. Indeed, within the striatum, the study of DA transmission, DA-

59 mediated signaling events and IEGs has been instrumental in shedding lights on the temporal
60 and spatial organization of striatal regions as well as on the functions of striatal cell types,
61 circuits and neuromodulatory systems [16–23]. However, whether and how multiple
62 (extracellular and intracellular) signaling pathways converge on the regulation of haloperidol-
63 induced IEGs in striatopallidal neurons remain to be fully understood.
64 Here, by using transgenic animals, pharmacological approaches and immunofluorescence
65 analyses, we investigated whether and how (*i*) non-DA neurotransmitters, notably glutamate
66 (NMDAR) and adenosine (A2aR), and (*ii*) intracellular PKA/DARPP-32 and mTOR signaling
67 pathways contribute to the regulation of haloperidol-induced IEGs in the striatum. We observed
68 that NMDAR and A2aR as well as PKA/DARPP-32 and mTOR pathways are distinctly
69 required and synergistically necessary to gate haloperidol-induced activation of striatopallidal
70 neurons. Our study, by further expanding the landscape of cellular and molecular modifications
71 elicited by antipsychotics, provide new evidence for the regulatory activity of striatopallidal
72 neurons.

Material and methods

Animals

8-12 weeks old mice were used. Male C57BL/6J mice (25–30 g) were purchased from Taconic (Tornbjerg, Denmark). Bacterial artificial chromosome transgenic mice expressing eGFP under the control of the promoter for the D2R (*Drd2*-eGFP) were generated by the GENSAT program (Gene Expression Nervous System Atlas) at the Rockefeller University [24] and back-crossed on a C57BL/6J background. Knock-in mice expressing a mutated form of DARPP-32, in which the threonine (Thr34) is replaced by an alanine (DARPP-32 T34A mutant mice) were generated as previously described [25] and back-crossed on a C57BL/6J background. Animals were maintained in a 12h light-dark cycle at a stable temperature of 22°C, with food and water *ad libitum*. Before any pharmacological experimentation, mice were handled and injected with saline for three consecutive days. The experiments were conducted in accordance with the guidelines of the Research Ethics Committee of Karolinska Institutet, Swedish Animal Welfare Agency and the 2010/63/EU directive for the care and use of experimental animals.

Drugs

Haloperidol (0.5 mg/kg, Sigma-Aldrich) was dissolved in saline containing 0.05% (v/v) acetic acid with the pH adjusted to 6.0 with 1M NaOH, and injected 60 min before perfusion. KW6002 (3 mg/kg), gift from Dr. Edilio Borroni (Hoffmann-LaRoche, Basel, Switzerland), was suspended by sonication in a solution of 5% (v/v) Tween 80 in saline and administered 5 min before haloperidol. MK-801 (0.1 mg/kg, Tocris) was dissolved in saline and administered 30 min before haloperidol. Rapamycin (5 mg/kg, LC Laboratories, Woburn, MA, USA) was dissolved in a solution of 5% (v/v) dimethylsulfoxide (DMSO), 5% Tween-20 and 15% (v/v) PEG-400, and administered (once per day) starting 3 days before the experiment. On the day

98 of the experiment, rapamycin was injected 45 min before haloperidol. All drugs were
99 administered intraperitoneally (i.p.) in a volume of 10 ml/kg, except for rapamycin, which was
100 administered in a volume of 5 ml/kg. When mice were not treated with drugs they received an
101 equivalent volume of the corresponding vehicle.
102
103 **Tissue preparation and immunofluorescence**
104 Tissues were prepared as previously described [17]. In brief, mice were rapidly anaesthetized
105 by i.p. injection of pentobarbital (500 mg/kg, Sanofi-Aventis, France) prior to intracardiac
106 perfusion of 4% (w/v) paraformaldehyde (PFA) in 0.1 M Na_2HPO_4/NaH_2PO_4 buffer (pH 7.5),
107 delivered with a peristaltic pump at 20 ml/min over 5 min. Brains were post-fixed overnight in
108 the same solution and stored at 4°C.
109 Thirty µm-thick sections were cut with a vibratome (Leica, France) and stored at -20°C in a
110 solution containing 30% (vol/vol) ethylene glycol, 30% (vol/vol) glycerol, and 0.1 M sodium
111 phosphate buffer, until they were processed for immunofluorescence.
112 Sections were processed as follows: free-floating sections were rinsed three times 10 minutes
113 in Tris-buffered saline (TBS, 50 mM Tris–HCL, 150 mM NaCl, pH 7.5). After 15 minutes of
114 incubation in 0.2% (vol/vol) Triton X-100 in TBS, sections were rinsed in TBS again and
115 blocked for 1 hour in a solution of 3% BSA in TBS. Sections were then incubated for 72 hours
116 at 4°C in 1% BSA, 0.15% Triton X-100 with the primary antibodies. Antibodies for cFos
117 (1:400, #sc-52), Arc (1:400, #sc-17839), and Zif268 (1:400, #sc-189) were purchased from
118 Santa Cruz Biotechnology [26]. GFP was amplified by using a chicken anti-GFP (1:1000,
119 Invitrogen). Sections were rinsed three times for 10 minutes in TBS and incubated for 45
120 minutes with goat Cy3-, Cy5-coupled (1:400, Jackson Immunoresearch) and/or goat A488
121 (1:400, Invitrogen) secondaries antibodies. Sections were rinsed for 10 minutes twice in TBS

122 and twice in Tris-buffer (1 M, pH 7.5) before mounting in a 1,4-diazabicyclo-[2. 2. 2]-octane
123 (DABCO, Sigma-Aldrich) solution.
124
125 **Confocal microscopy and image analysis**
126 Single and/or double-immunolabeled images from each region of interest were obtained using
127 sequential laser scanning confocal microscopy (Zeiss LSM510 META). Photomicrographs
128 were obtained with the following band-pass and long-pass filter setting: GFP (band pass filter:
129 505-530), Cy3 (band pass filter: 560-615) and Cy5 (long-pass filter 650).
130 Quantifications were performed in 325.75 µm × 325.75 µm confocal images.
131 Immunofluorescent cells were counted blindly of the treatment/group by using the cell counter
132 plugin of the ImageJ software taking as standard reference a fixed threshold of fluorescence.
133
134 **Statistical analysis**
135 All statistical comparisons were performed with two-sided tests in Prism 6 (GraphPad Software,
136 La Jolla, CA, USA). The distribution of data was determined with Shapiro-Wilk normality test.
137 No sample size calculations were performed. To determine outliers in every experimental group
138 we performed the Grubbs' test in Prism. No outliers were identified. All the data were analyzed
139 using either Student *t*-test with equal variances or One-way ANOVA. In all cases, significance
140 threshold was automatically set at $p < 0.05$. The One-way ANOVA analysis, where treatment
141 was the independent variable, was followed by Bonferroni *post hoc* test for specific
142 comparisons only when overall ANOVA revealed a significant difference (at least $p < 0.05$)
143 among groups. All the values and statistical analyses are reported in the figure legends.

144 **Results**

145

146 **Haloperidol induces contrasting cell type-specific regulation of IEGs in the dorsal**
147 **striatum**

148 It is well-established that haloperidol induces the expression of the IEGs cFos, Arc and Zif268
149 in the striatum [27–32]. Here, we took advantage of *Drd2*-eGFP mice, to visualize
150 striatopallidal D2R$^+$-neurons and revisit the cell type-specific expression patterns of cFos, Arc
151 and Zif268 induced by haloperidol. As expected, an increased number of cFos-, Arc- and
152 Zif268-immunoreactive neurons was observed in the dorsal striatum 60 min after a single
153 administration of haloperidol (0.5 mg/kg, ip) (**Fig. 1A**). Cell type-specific analysis revealed that
154 cFos and Arc were exclusively triggered in striatopallidal D2R$^+$-neurons (**Fig. 1A, B**). In
155 contrast, Zif268 was increased in both striatonigral (D2R$^-$-neurons) and striatopallidal D2R$^+$-
156 neurons (**Fig. 1A, B**), although Zif268$^+$/D2R$^+$-neurons outnumbered Zif268$^+$/D2R$^-$-neurons
157 (**Fig. 1A, B**). This first set of results indicates that cFos and Arc, but not Zif268, can be used as
158 *bonafide* markers to study long-term cellular and molecular events induced by haloperidol in
159 striatopallidal neurons.

160 Next, we wondered whether the induction of cFos and Arc spatially occurred in the same
161 or different subpopulations of D2R$^+$-neurons. Thus, we performed a double
162 immunofluorescence analysis for cFos and Arc. We observed a complete colocalization (341
163 out of 341 neurons, 100%) of cFos with Arc (**Fig. 2A, B**). However, we also observed that,
164 among all Arc$^+$-neurons, 23.5% (105 out of 446 neurons) did not express cFos (**Fig. 2A, B**),
165 therefore indicating that haloperidol-induced cellular modifications may occur at different
166 spatial scales.

167

168 **Haloperidol-induced cFos and Arc require the activation of A2a and NMDA receptors**

169 Next, we investigated the contribution of non-DA receptors in the regulation of haloperidol-
170 induced cFos and Arc (**Fig. 1**). We focused on A2a [highly enriched in striatopallidal neurons,
171 [33]] and NMDA receptors as they strongly contribute to the regulation of the striatopallidal
172 neurons' activity and plasticity [34,35].
173 We observed that pretreatment with the specific A2aR antagonist KW-6002 [also known as
174 istradefylline, 3 mg/kg, [8]] significantly reduced haloperidol-induced cFos expression in the
175 striatum (**Fig. 3A, B**). In addition, when mice were pretreated with the NMDAR antagonist
176 MK-801 (also known as dizocilpine, 0.1 mg/kg) we noticed a dramatic reduction in haloperidol-
177 induced cFos activation (**Fig. 3A, B**). However, both A2aR and NMDAR blockades failed in
178 fully preventing cFos expression in haloperidol-treated mice. Interestingly, co-administration
179 of KW-6002 and MK-801 before haloperidol injection resulted in a complete loss of cFos
180 activation (**Fig. 3A, B**). Similar regulatory mechanisms were observed when Arc was used as
181 molecular proxy of haloperidol-induced activation of striatopallidal neurons. In fact, while KW-
182 6002 and MK-801 distinctly reduced haloperidol-induced Arc expression (**Fig. 3C, D**), co-
183 administration of both A2aR and NMDAR antagonists completely prevented the
184 cellular/molecular response of striatopallidal neurons to haloperidol (**Fig. 3C, D**).
185 These results indicate that ambient adenosine and glutamate are necessary and act
186 synergistically to gate the responsiveness of striatopallidal neurons to the antipsychotic
187 haloperidol.
188
189 **Haloperidol-induced cFos and Arc require the involvement of PKA/DARPP-32 and**
190 **mTOR pathways**
191 Haloperidol promotes the PKA-dependent phosphorylation of DARPP-32 on Thr34 which
192 converts DARPP-32 into an inhibitor of protein phosphatase 1 (PP-1), thereby inhibiting the
193 dephosphorylation of numerous cAMP/PKA-dependent molecular targets [36]. Indeed, at the

194 intracellular level, A2aR and D2R oppositely regulate the cAMP/PKA/DARPP-32 pathway [7]
195 and seminal reports have shown that the cellular modifications elicited by haloperidol depend
196 on the recruitment of key intracellular signaling cascades such as the PKA/DARPP-32 and
197 mTOR pathways [7–9,37]. Here, we decided to investigate whether these two pathways were,
198 distinctly and/or synergistically, involved in the regulation of haloperidol-induced IEGs.
199 To dampen the PKA/DARPP-32 striatal path *in vivo*, we used DARPP-32 mutant mice in which
200 Thr34 was replaced by Ala (T34A) [25]. Compared to WT mice, T34A mutant mice showed
201 reduced cFos and Arc activation following haloperidol administration (**Fig. 4A-D**). To inhibit
202 the mTOR signaling pathway, mice were pre-treated with rapamycin (5 mg/kg, [9]). As shown
203 in **Fig. 4**, rapamycin reduced haloperidol-induced cFos and Arc (**Fig. 4A-D**). Interestingly,
204 when both PKA/DARPP-32 and mTOR pathways were downregulated (rapamycin
205 administration to T34A mutant mice), we observed a full abolishment in the induction of cFos
206 and Arc following haloperidol administration.
207 These results indicate that intracellular cAMP/PKA and mTOR pathways are both
208 synergistically mobilized and required for the full cellular/molecular action of haloperidol onto
209 striatopallidal neurons.

210 **Discussion**

211 In this study we show that the typical antipsychotic haloperidol, which alters the activity and
212 plasticity of D2R-MSNs [38,39], elicits cell type-specific activation of IEGs in the mouse
213 striatum (D1-MSNs *vs* D2R-MSNs) and that this activation requires the contribution of
214 different signaling actors. Here, we focused on cFos, Arc and Zif268 which, by representing
215 the result of long-term cellular/molecular adaptations, are dynamically triggered by a plethora
216 of stimuli and are also involved in the regulation of several neuronal functions [40–43]. We
217 observed that haloperidol-induced activation of the IEGs cFos and Arc was exclusively
218 restricted to striatopallidal $D2R^+$-neurons, whereas Zif268 expression was triggered in both
219 striatonigral and striatopallidal neurons. These findings are in line with and further extend
220 previous observations on haloperidol-regulated phospho-proteins (histone H3, ribosomal
221 protein S6), highlighting the cell type-specific reactivity of MSNs to DA-related agents
222 [7,8,18,19]. However, the cell type-unspecific expression of Zif268 in the striatal MSNs
223 suggests that haloperidol, most likely through an indirect mechanism involving striatal
224 cholinergic and/or GABAergic interneurons [44,45], may also regulate the activity of D1R-
225 MSNs even though no major changes in the electrophysiological profile of this cell type have
226 been observed [39].

227 In addition to the selective induction of cFos and Arc in striatopallidal neurons following
228 haloperidol administration, we noticed that $cFos^+/D2R^+$-neurons seemed to represent a sub-
229 population of $Arc^+/D2R^+$-neurons. This is of interest in light of recent reports describing that
230 the two major striatal cell populations (D1R- and D2R-MSNs) may actually consist of different
231 subpopulations with distinct spatiocellular and spatiomolecular features [46–49]. Indeed, the
232 use of unbiased high-throughput technologies, either at the single cell or population level, will
233 be instrumental to reappraise the functional heterogeneity of striatal neurons.

234 The therapeutic action as well as the side effects of antipsychotics strongly depend on the
235 pharmacological blockade of D2R. However, other neurotransmitters participate in regulating
236 the cellular activity of D2R-containing neurons and consequently scale the behavioral effects
237 of haloperidol (i.e. catalepsy). Adenosine and glutamate represent two of the major
238 neuromodulators/neurotransmitters able to regulate the activity of MSNs and seminal reports
239 have shown that their respective receptors, A2aR and NMDAR, distinctly influences
240 haloperidol-associated effects. In fact, pharmacological administration of A2aR antagonists
241 and/or genetic deletion of A2aR counteract haloperidol-associated behaviors [50–52]. This is
242 in line with our observation showing that blockade of A2aR was sufficient to partially blunt
243 haloperidol-induced cFos and Arc expression in the striatum. This action is mainly due to the
244 opposite regulation exerted on D2R-MSNs by A2aR and D2R which are positively (via G_s) and
245 negatively (via G_i) coupled to the adenylyl cyclase, respectively. While the A2aR and D2R
246 downstream signaling events may buffer the intracellular production of cAMP, several reports
247 have also indicated that A2aR and D2R can heterodimerize (protein-protein interaction) at the
248 membrane levels, therefore influencing the activity of each receptor [53–58].
249 Beside ambient striatal adenosine, the excitatory neurotransmitter glutamate, mostly released
250 from cortico-striatal and thalamo-striatal projections, is another key player in the regulation of
251 MSNs. Seminal studies have shown that pharmacological blockade of NMDAR was sufficient
252 to blunt haloperidol-induced catalepsy [59–61] and our study provide further evidence that
253 NMDARs are required for haloperidol-induced cFos [62,63] and Arc in the striatum. Indeed,
254 NMDARs can gate the action of haloperidol (D2R) through multiple mechanisms. First,
255 activation of NMDAR can boost the activation of the cAMP/PKA cascade via an indirect
256 A2aR-dependent mechanism [64]. Second, haloperidol-induced cAMP/PKA activity can lead
257 to the PKA-dependent phosphorylation of NMDAR subunits, thus facilitating glutamatergic
258 transmission [65]. Third, recent reports have shown that dopamine receptors can also

259 heterodimerize with NMDARs [66–68]. Forth, blockade of NMDAR may also counteract the
260 D2R-dependent modulation of cortico-striatal glutamate transmission [69,70]. Therefore,
261 NMDAR antagonists may blunt haloperidol-elicited striatal IEGs through all these
262 mechanisms.
263 However, our study also shows that while blockade of A2aR or NMDAR blunted haloperidol-
264 induced IEGs, co-administration of A2aR and NMDAR antagonists (KW-6002 and MK-801,
265 respectively) resulted in the full abolishment of cFos and Arc. These results indicate that to
266 modulate striatal networks haloperidol requires the dynamic and synergistic action of dopamine
267 (D2R), adenosine (A2aR) and glutamate (NMDAR).
268 At the intracellular molecular level, haloperidol mobilizes the PKA/DARPP-32 cascade as well
269 as the mTOR signaling pathway [7–9,37,71,72]. Indeed, downregulation of the PKA/DARPP-
270 32 [73] and mTOR [72] pathways dampened haloperidol-induced catalepsy. In line with these
271 reports, we observed a reduction of cFos and Arc when haloperidol was administered to
272 DARPP-32 T34A mutant mice (inhibition of PKA/DARPP-32 cascade) and also when it was
273 preceded by the injection of rapamycin (inhibition of mTOR). However, we also noticed that
274 when both signaling paths were downregulated (rapamycin administration to T34A mice)
275 haloperidol completely failed to induce cFos and Arc expression in the striatum, therefore
276 indicating that both signaling cascades are necessary and required for the full action of
277 haloperidol. It is interesting to note that, beside the antagonistic functions of A2aR and D2R on
278 the cAMP/PKA/DARPP-32 pathway, NMDAR have also been associated to the downstream
279 activation of the mTOR pathway [74–76], thus indicating that the converging contribution of
280 these two signaling cascades may actually reflect the dynamic involvement of adenosine and
281 glutamate in mediating the D2R-dependent action of haloperidol in striatopallidal neurons.
282

In conclusion, our study, by confirming and expanding previous observations and also by providing a new synergic perspective of striatal regulatory mechanisms, highlights the heterogenous complexity of *in vivo* processes which may be involved in the therapeutic and adverse effects of antipsychotics.

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Conflicts of Interest

The authors declare no conflicts of interest.

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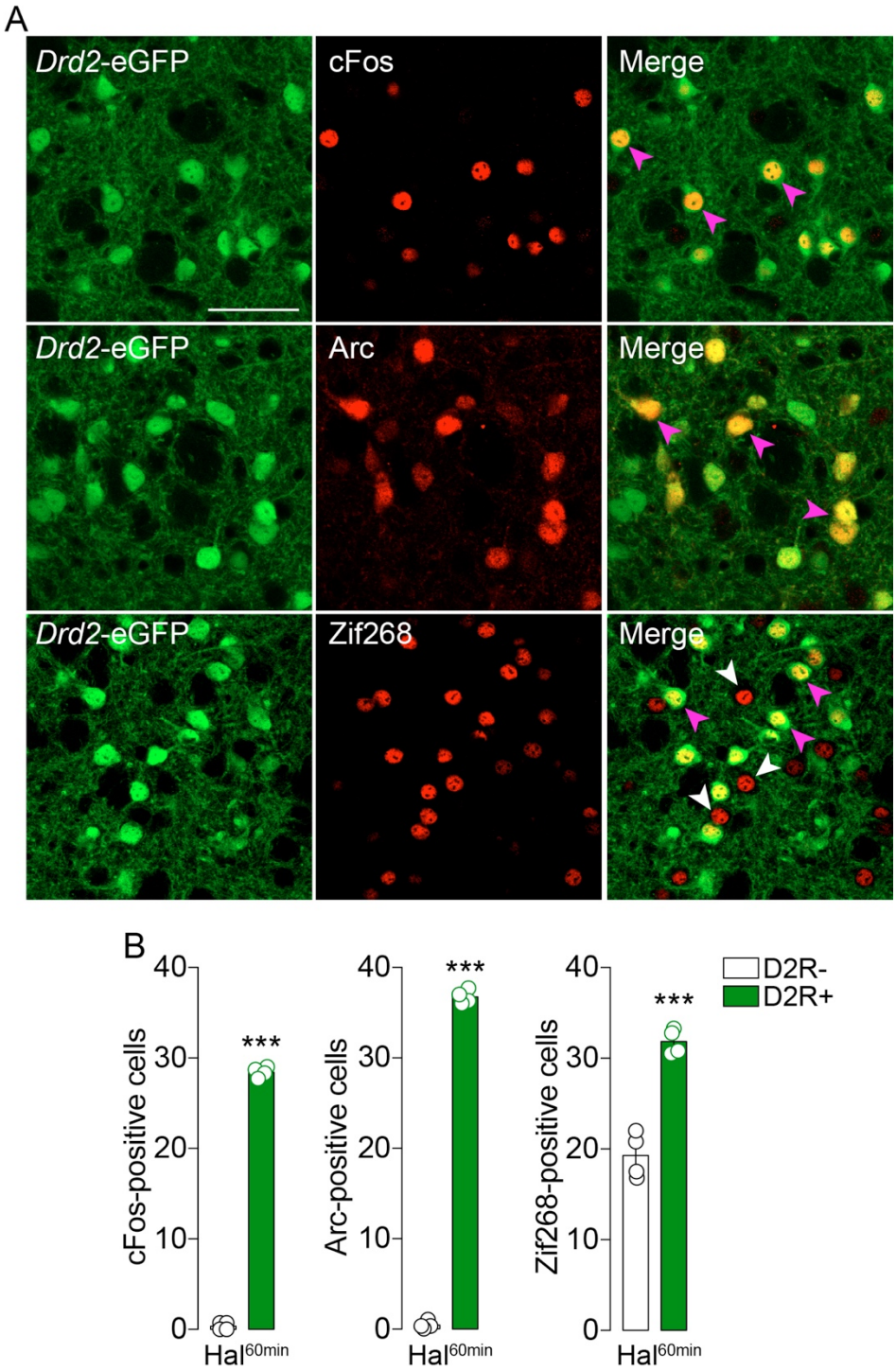
570

571 **Figures and Legends**

Figure 1



572

573 **Figure 1: Haloperidol induces cFos, Arc and Zif268 in a cell type-specific manner in the**

574 **mouse striatum.**

575 (**A**) Double immunofluorescence detection of cFos, Arc and Zif268 in the striatum of *Drd2*-
576 eGFP mice 60 minutes after haloperidol administration. Arrows in magenta indicate the
577 expression of immediate early genes (IEGs) in $D2R^+$-neurons, whereas arrows in white indicate
578 expression of IEGs in $D2R^-$-neurons. Scale bar: 50 μm. (**B**) Quantification of cFos, Arc and
579 Zif268 in both $D2R^+$- and $D2R^-$-neurons. Statistics: *** $p < 0.001$, Student's t-test (n=4/group):
580 $t = 81.12$, $df = 6$ (cFos); $t = 83.58$, $df = 6$ (Arc); $t = 8.72$, $df = 6$ (Zif268).
581

Figure 2

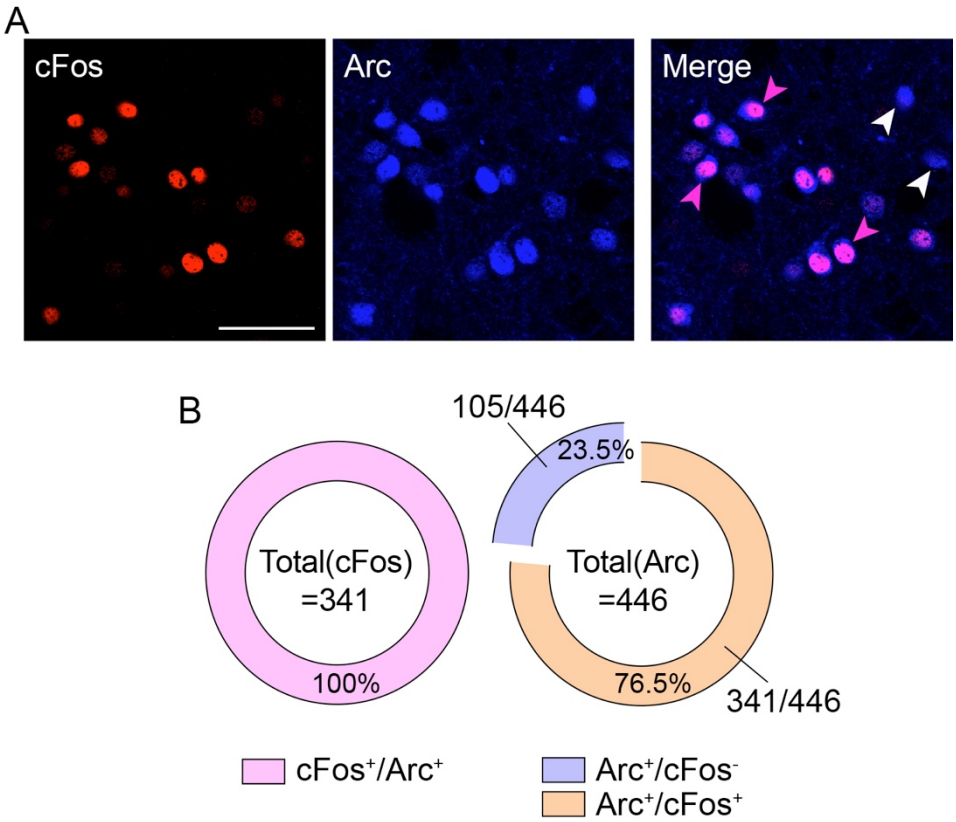


Figure 2: Spatiocellular organization of haloperidol-induced cFos and Arc.

(**A**) Double immunofluorescence detection of cFos (red) and Arc (blue) following haloperidol administration. Scale bar: 50 μm. (**B**) Quantification of the degree of colocalization between cFos and Arc. Note that 23.5% of Arc^{+}-neurons did not express cFos.

Figure 3

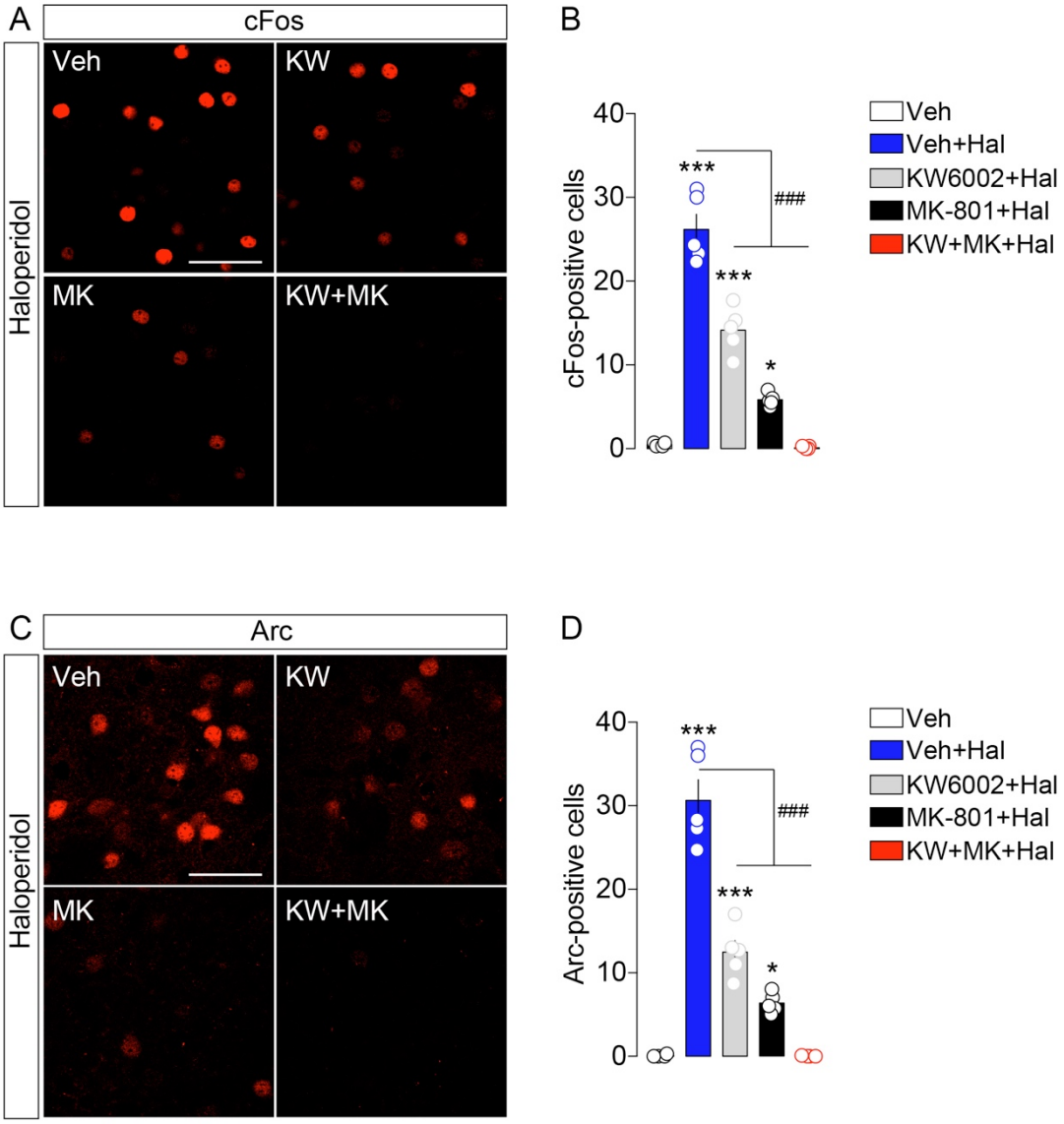
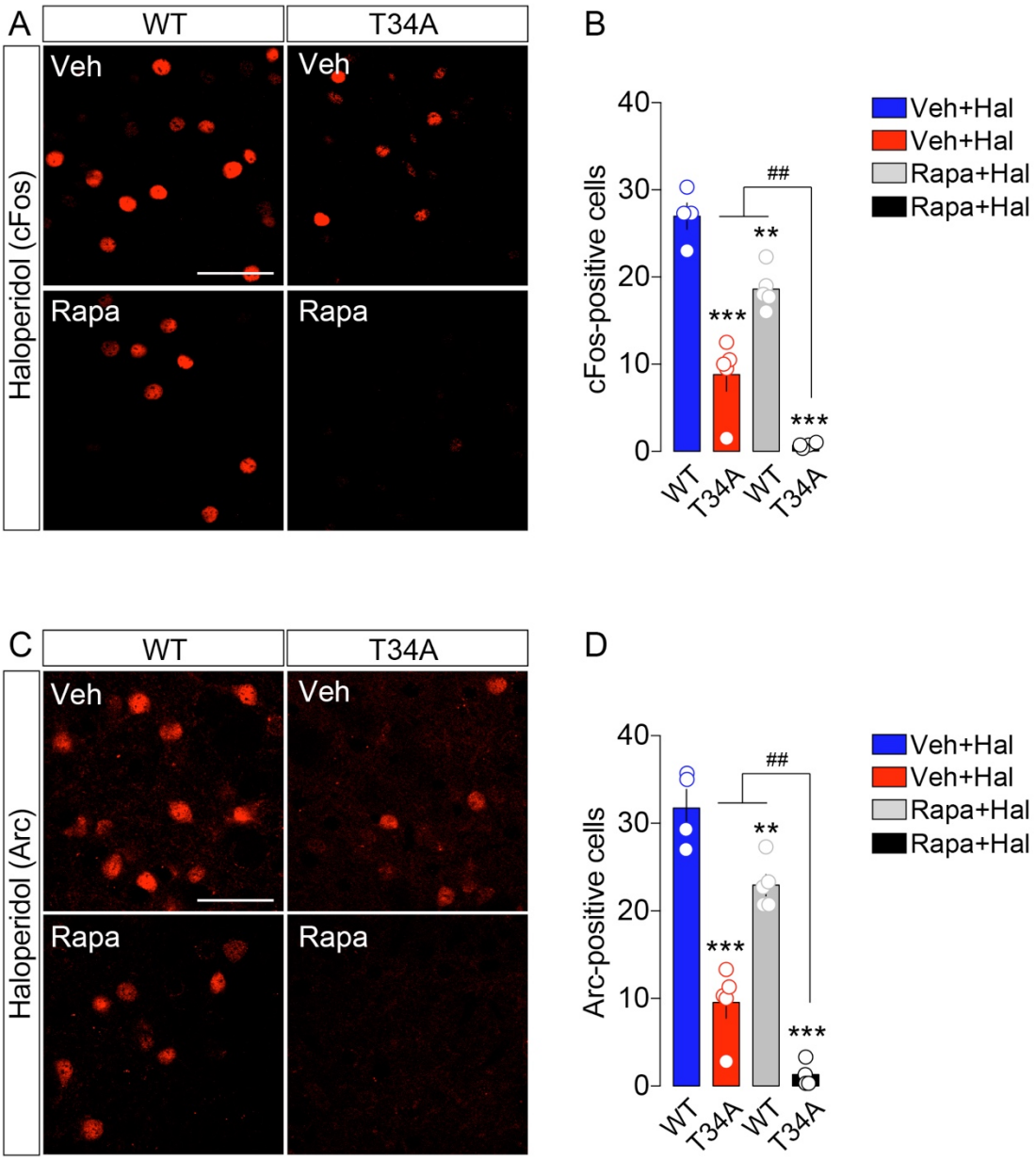


Figure 3: Haloperidol-induced cFos and Arc require the activation of A2a and NMDA receptors.

(**A**) Immunofluorescence detection of cFos in the striatum of animals treated with vehicle (n=4), haloperidol (n=5), KW-6002+haloperidol (n=5), MK-801+haloperidol (n=5) and KW-6002+MK-801+haloperidol (n=5). Scale bar: 50 µm. (**B**) Quantification of cFos^{+}-neurons. Statistics: *p<0.05 and ***p<0.001 (drugs vs vehicle), $^{###}$p<0.001 (KW-6002+haloperidol and MK-801+haloperidol *vs* haloperidol). One-way ANOVA: $F_{(4,\ 19)}$=113.9, p<0.0001. (**C**)

596 Immunofluorescence detection of Arc in the striatum following pharmacological
597 administrations. Scale bar: 50 μm. (**D**) Quantification of Arc^{+}-neurons. Statistics: *$p<0.05$ and
598 ***$p<0.001$ (drugs vs vehicle), $^{###}p<0.001$ (KW-6002+haloperidol and MK-801+haloperidol
599 *vs* haloperidol). One-way ANOVA: $F_{(4, 19)}=90.46$, $p<0.0001$.
600

Figure 4



601

602 **Figure 4: Haloperidol-induced cFos and Arc require the intracellular activation of**

603 **PKA/DARPP-32 and mTOR pathways.**

604 (**A, C**) Immunofluorescence detection of cFos (**A**) and Arc (**C**) in the striatum of WT and T34A

605 animals treated with vehicle+haloperidol, or rapamycin+haloperidol. Scale bars: 50 µm. (**B**)

606 Quantification of cFos^{+}-neurons in WT and T34A mutant mice treated with vehicle+haloperidol

607 and rapamycin+haloperidol (n=4-5/group). Statistics: **p<0.01 and ***p<0.001 (T34A+Halo,

608 WT+Rapa+Hal, T34A+Rapa+Hal *vs* WT+Hal), $^{\#\#}p<0.01$ (T34A+Rapa+Hal *vs* T34A+Hal and
609 WT+Rapa+Hal). One-way ANOVA: $F_{(3, 14)}=62.67$, $p<0.0001$. (**D**) Quantification of Arc^{+}-
610 neurons in WT and T34A mutant mice treated with vehicle+haloperidol and
611 rapamycin+haloperidol (n=4-5/group). Statistics: $^{**}p<0.01$ and $^{***}p<0.001$ (T34A+Hal,
612 WT+Rapa+Hal, T34A+Rapa+Hal *vs* WT+Hal), $^{\#\#}p<0.01$ (T34A+Rapa+Hal *vs* T34A+Hal and
613 WT+Rapa+Hal). One-way ANOVA: $F_{(3, 14)}=70.07$, $p<0.0001$.