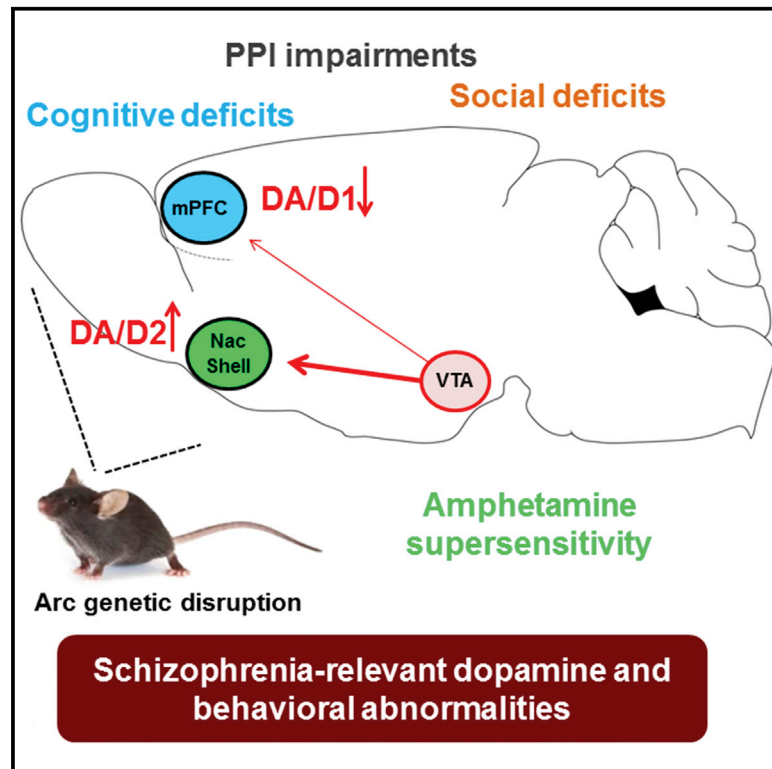


Genetic Disruption of Arc/Arg3.1 in Mice Causes Alterations in Dopamine and Neurobehavioral Phenotypes Related to Schizophrenia

Graphical Abstract



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In Brief

Managò et al. find that, consistent with schizophrenia-related phenotypes, disruption of Arc in mice produces deficits in sensorimotor gating, cognitive functions, social behaviors, and amphetamine-induced psychomotor responses. Furthermore, genetic disruption of Arc leads to concomitant hypoactive mesocortical and hyperactive mesostriatal dopamine pathways.

Highlights

- Arc genetic disruption recapitulates schizophrenia-relevant behavioral abnormalities
- Arc disruption leads to hypoactive PFC dopamine (DA) and hyperactive striatal DA system
- Hypo-PFC and hyper-striatal DA mediates cognitive and motor changes, respectively
- Arc is a convergence point for genes implicated in schizophrenia risk

Genetic Disruption of Arc/Arg3.1 in Mice Causes Alterations in Dopamine and Neurobehavioral Phenotypes Related to Schizophrenia

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SUMMARY

Human genetic studies have recently suggested that the postsynaptic activity-regulated cytoskeleton-associated protein (Arc) complex is a convergence signal for several genes implicated in schizophrenia. However, the functional significance of Arc in schizophrenia-related neurobehavioral phenotypes and brain circuits is unclear. Here, we find that, consistent with schizophrenia-related phenotypes, disruption of Arc in mice produces deficits in sensorimotor gating, cognitive functions, social behaviors, and amphetamine-induced psychomotor responses. Furthermore, genetic disruption of Arc leads to concomitant hypoactive mesocortical and hyperactive mesostriatal dopamine pathways. Application of a D1 agonist to the prefrontal cortex or a D2 antagonist in the ventral striatum rescues Arc-dependent cognitive or psychomotor abnormalities, respectively. Our findings demonstrate a role for Arc in the regulation of dopaminergic neurotransmission and related behaviors. The results also provide initial biological support implicating Arc in dopaminergic and behavioral abnormalities related to schizophrenia.

INTRODUCTION

Schizophrenia is a devastating disorder strongly related to genetic factors (Sullivan et al., 2003). This debilitating disorder is defined by behavioral symptoms that encompass a large spectrum of abnormalities, including cognitive and sensorimotor deficits, social withdrawal, and hallucinations. Although the neurobiological mechanisms underlying these behavioral abnor-

malities remain unclear, the pathophysiology of schizophrenia, its treatments, and aspects of its associated behavioral deficits have been consistently linked with a dysregulation of dopaminergic, glutamatergic, and GABAergic neural transmission, especially in brain areas such as the prefrontal cortex (PFC) and striatum (Howes and Kapur, 2009; Simpson et al., 2010; Winterer and Weinberger, 2004).

Recent large-scale genetic studies of schizophrenia have noted that many relevant variants involve genes encoding proteins interacting with the activity-regulated cytoskeletal-associated (Arc) postsynaptic signaling complexes (Fromer et al., 2014; Purcell et al., 2014; Huentelman et al., 2015). Arc chromosomal microdeletion and intragenic SNPs have also been found in association with neurodevelopmental disorders such as schizophrenia (Hu et al., 2015; Huentelman et al., 2015). In addition, reduced expression of Arc mRNA has been detected in the PFC of individuals with schizophrenia (Guillozet-Bongaarts et al., 2014). Despite this consistent evidence pointing to convergence on Arc, the neurobiological implication of Arc in schizophrenia-related behavioral phenotypes and brain systems remains unclear.

Arc is a neural activity-regulated immediate early gene that is expressed selectively in Ca²⁺/calmodulin-dependent kinase II alpha (CaMKIIa)-expressing neurons in the neocortex, hippocampus, and striatum (Miyashita et al., 2008; Vazdarjanova et al., 2006). Extensive studies have shown that the Arc protein is functionally involved in long-lasting forms of synaptic plasticity (Plath et al., 2006; Shepherd et al., 2006; Rial Verde et al., 2006; Waung et al., 2008; Park et al., 2008; Jakkamsetti et al., 2013). In particular, Arc has been reported to regulate synaptic activities and neuronal firing patterns through AMPA and NMDA-type glutamate receptors (Chowdhury et al., 2006; Zhang et al., 2015). Furthermore, genetic disruptions of the Arc gene have been shown to impair experience-dependent cortical circuit functions (McCurry et al., 2010; Wang et al., 2006) and consolidation of memories (Plath et al., 2006; Ren et al., 2014; Cao

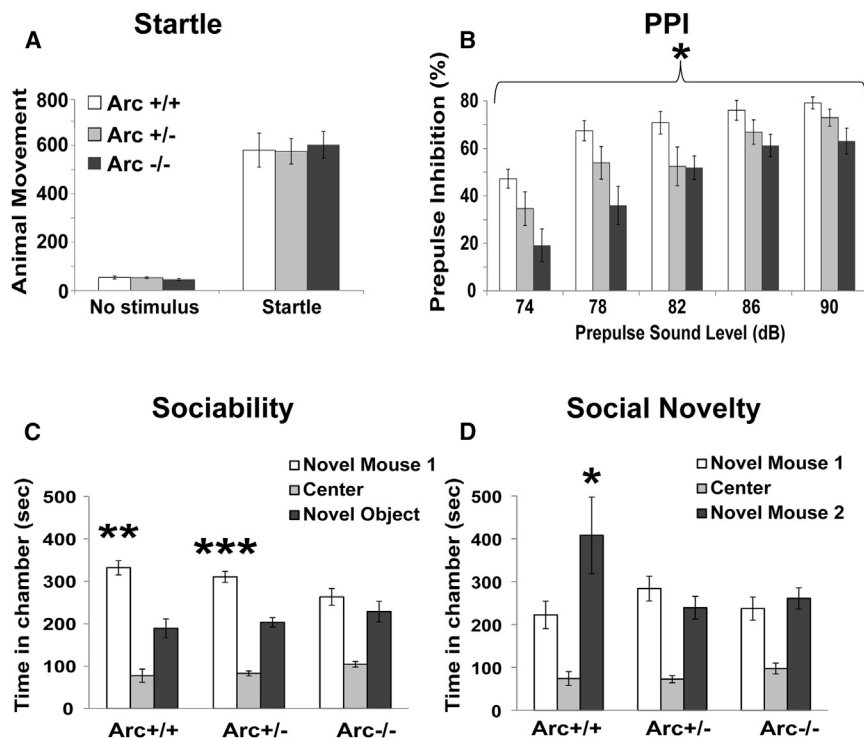


Figure 1. Impaired Sensorimotor Gating and Social Abilities in Arc Knockout Mice

(A) Animal movements (a.u.) displayed by Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates following the presentation of no stimulus or a 120 dB acoustic stimulus (Startle).

(B) Percent PPI of the acoustic startle response displayed by the same mice after the presentation of 74, 78, 82, 86, and 90 dB prepulse stimuli. n = 20–26/group. *p < 0.05, Arc^{-/-} versus Arc^{+/+}.

(C and D) Time spent in each compartment of the three-chambered arena during (C) the sociability and (D) the social novelty tests displayed by Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates. n = 9–17/group. ***p < 0.0005, **p < 0.005, and *p < 0.05 versus the novel object or the novel mouse within the same genotype. Values represent mean ± SEM throughout all figures.

RESULTS

Arc Genetic Disruption Impaired Prepulse Inhibition Abilities, but Not Startle Responses

Prepulse inhibition (PPI) is a sensorimotor gating measure that is attenuated in patients with schizophrenia (Swerdlow

et al., 2006), is highly conserved across mammalian species, and can be studied experimentally in rodents (Papaleo et al., 2012).

Reduction (+/-) or absence (-/-) of Arc did not affect general health or physical abilities (Table S1; Supplemental Results). Arc genotype did not affect acoustic startle reactivity or basal activity ($F_{2,64} = 0.03$, $p = 0.97$; Figure 1A). Instead, PPI showed a genotype effect ($F_{2,64} = 4.46$, $p < 0.02$; Figure 1B), with Arc^{-/-} mice displaying disrupted PPI ($p = 0.008$) and Arc^{+/-} mice a tendency ($p = 0.08$) compared to Arc^{+/+} littermates. These results indicate that genetic modifications reducing Arc decrease sensorimotor gating abilities, consistent with PPI deficits in schizophrenia.

Arc Genetic Disruption Impaired Social Abilities

Disrupted social behaviors are characteristic features in schizophrenia, embedded in so-called negative symptoms (Millan et al., 2014). We thus assessed Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates in a well-validated social approach task (Papaleo et al., 2011).

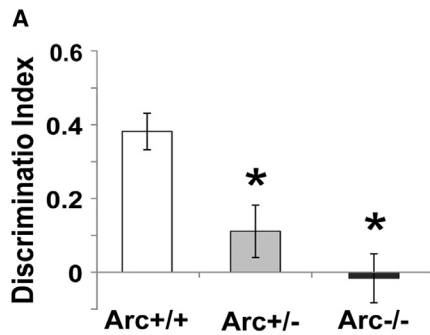
Sociability, defined as spending more time in the chamber with a novel mouse than in the chamber with a novel object, was evident in both Arc^{+/+} ($F_{1,8} = 15.58$, $p < 0.005$) and Arc^{+/-} ($F_{1,16} = 22.31$, $p < 0.0005$; Figure 1C) mice. In contrast, Arc^{-/-} mice presented sociability deficits as they spent the same amount of time with the novel mouse and the novel object ($F_{1,5} = 0.63$, $p = 0.46$; Figure 1C). Thus Arc deletion completely abolished social preference versus a conspecific compared to an inanimate object.

Deficits in social novelty recognition were also detected in Arc mutant mice. Arc^{+/+} mice spent more time in the chamber containing the newly introduced mouse (novel mouse 2) than in the chamber containing the now familiar mouse ($F_{1,8} = 7.65$, $p < 0.05$; Figure 1D). In contrast, both Arc^{+/-} and Arc^{-/-} mice

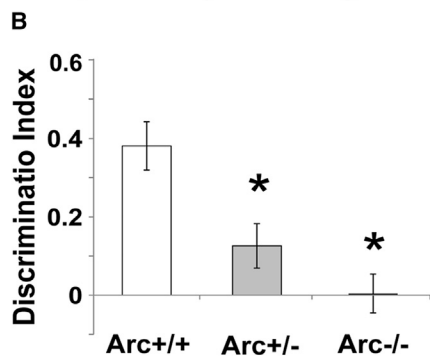
et al., 2015). Although these results highlight a central role of Arc in brain plasticity and in the regulation of two types of glutamatergic receptors (AMPA and NMDA) that have been implicated in the pathophysiology of schizophrenia, whether genetic disruption of Arc will lead to schizophrenia-related behavioral and brain system deficits is unknown.

Using genetically modified mice with targeted deletion of the Arc gene, we aimed to investigate the effects of Arc genetic disruptions on behavioral functions that have been studied in rodents as relevant to schizophrenia. We did not attempt to find a mouse model of an entire psychiatric disorder but rather focused on disease-related functional dimensions of behavior. Particularly, because one of the long-standing pathophysiological hypotheses of schizophrenia involves a dysregulated dopaminergic system (Weinstein et al., 2016), we investigated the effects of Arc genetic disruption on different aspects of dopaminergic system functions. We found that Arc gene disruption leads to behavioral abnormalities comprising a broad range of cognitive, negative and positive schizophrenia-like symptoms. In addition, using a combination of ex vivo molecular assessments, in vivo microdialysis, and electrical stimulation coupled with in vivo two-photon imaging, we show that Arc genetic disruption leads to divergent alterations between the PFC and striatal dopaminergic system that capture aspects of schizophrenia-related neuropathophysiology. Specifically, reduced Arc gene expression resulted in a hypo-dopaminergic response in the PFC and increased dopamine/D2 signaling in the striatum. These data provide initial biological support that abnormal Arc function may cause dysfunctions in the dopaminergic system and behavioral abnormalities related to schizophrenia.

Temporal Order Object Recognition



Spatial Object Recognition



Novel Object Recognition

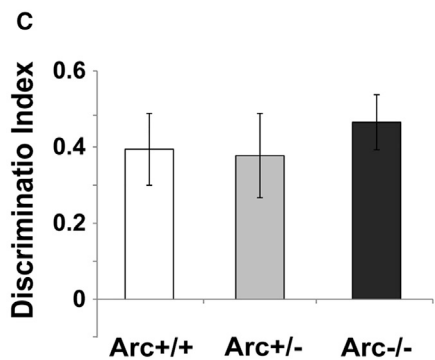


Figure 2. Impaired Temporal Order and Spatial Object Recognition Memory in Arc Knockout Mice

Discrimination ratio displayed by Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates (A) during the 5-min temporal order object recognition test (n = 18–20/group), (B) during the 5-min spatial object recognition test (n = 17–22/group), and (C) during the 5-min novel object recognition test (n = 6–12/group). *p < 0.01 versus Arc^{+/+} mice.

spent the same amount of time between the newly introduced and familiar mice ($F_{1,16} = 0.67$, $p = 0.43$ and $F_{1,5} = 0.22$, $p = 0.66$ for Arc^{+/-} and Arc^{-/-} mice, respectively; Figure 1D). For both tests, entries into the left and right side chambers did not differ within each genotype or across genotypes (Figure S1). No innate side preference was observed across genotypes before the start of the testing. These data indicate that genetic

mutations reducing Arc disrupt social discrimination abilities, consistent with similar deficits in schizophrenia.

Arc Genetic Disruption Produced Schizophrenia-Relevant Cognitive Impairments

Cognitive deficits have been suggested as core symptoms of schizophrenia. We tested Arc genetically modified mice in a temporal order object recognition task that measures recency discrimination, a cognitive ability disrupted in patients with schizophrenia (Rizzo et al., 1996; Schwartz et al., 1991).

An Arc genotype effect was evident during the test phase ($F_{2,54} = 9.86$; $p < 0.0002$; Figure 2A). In particular, the performance of Arc^{+/-} and Arc^{-/-} mice was significantly worse than that of Arc^{+/+} mice ($p < 0.005$). While Arc^{+/+} mice were spending more time exploring the object presented least recently, Arc^{+/-} and Arc^{-/-} mice failed to show any preference for the less recent object. Thus, Arc genetic reduction impaired cognitive functions dependent on the medial prefrontal cortex (mPFC), hippocampus, and perirhinal cortex (PRH; Barker and Warburton, 2011).

To better dissect the neuroanatomical components of Arc-dependent cognitive deficits, we tested two other cohorts of naive mutants in a spatial object recognition task, exclusively dependent on hippocampal functions, and in a novel object recognition task that depends on the PRH, but not on the mPFC or the hippocampus (Barker et al., 2007; Barker and Warburton, 2011). In the spatial object recognition task, there was an Arc effect ($F_{2,54} = 10.66$, $p < 0.002$; Figure 2B). In contrast to Arc^{+/+} mice, Arc^{-/-} and Arc^{+/-} mice were not able to recognize which object was displaced ($p < 0.003$). These results indicate that Arc is fundamental for spatial memory functions.

The novel object recognition task revealed no Arc effects ($F_{2,24} = 0.16$; $p = 0.85$; Figure 2C). As well as Arc^{+/+} mice, Arc^{+/-} and Arc^{-/-} mice spent more time exploring the novel object compared to the familiar one. This indicates unimpaired novel-familiar object discrimination abilities and unaltered PRH functioning. In all three experiments, Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} mice spent equal amount of time exploring the objects in every phase of the task (Figure S2). Thus, Arc genetic reduction did not alter motivation, curiosity, motor, olfactory, tactile, or visual functions that might affect object recognition.

Overall, these results indicate that the Arc-dependent cognitive deficits might derive from a dysfunctional mPFC and/or hippocampus, two brain regions implicated in the cognitive deficits found in schizophrenia (Dreher et al., 2012; Rasetti et al., 2014).

Arc Genetic Disruption Produced Amphetamine Supersensitivity

Amphetamine exacerbates psychotic experiences in patients with schizophrenia and can be psychogenic in normal human subjects (Laruelle et al., 1999). We thus tested Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates in an open field arena in basal conditions and following amphetamine injection.

A genotype × time interaction effect was detected in the basal locomotor activity during the first day ($F_{22,572} = 1.98$, $p < 0.005$). All three genotypes decreased the distance traveled in the open field arena over time. However, Arc^{-/-} mice were slightly more active than Arc^{+/-} and Arc^{+/+} mice during the first 5 min ($p < 0.05$; Figure 3A).

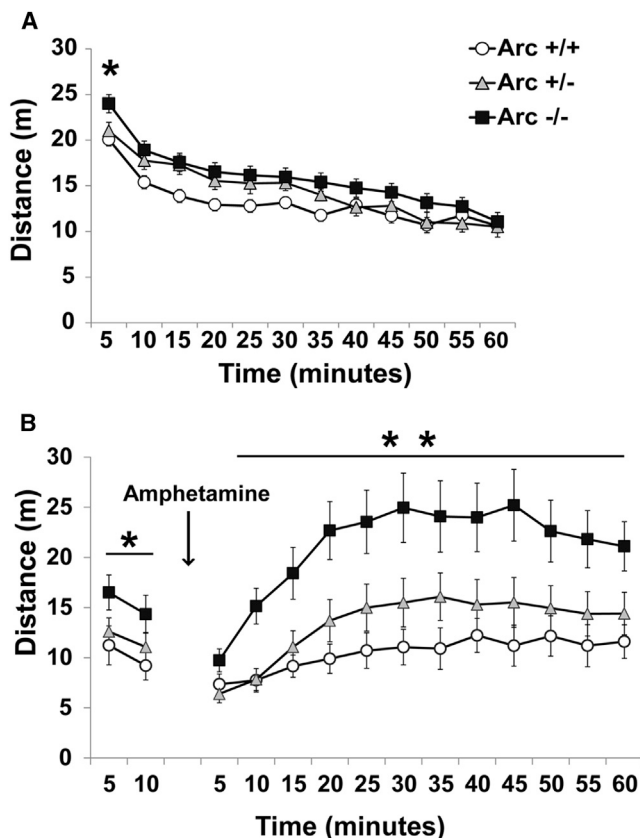


Figure 3. Amphetamine Supersensitivity in Arc Knockout Mice

(A) Ambulatory distance displayed by Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates during the first exposure to the empty open field arena (n = 14–24/group). *p < 0.05 versus Arc^{+/+} within the same time point.

(B) Ambulatory distance displayed by Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} mice during the 10 min before and 60 min after the amphetamine injection (1.5 mg/kg, i.p.) (n = 13–20/group).

*p < 0.01 and **p < 0.001 versus Arc^{+/+} and Arc^{+/-}.

Two days later, a genotype effect was present again during the first 10 min in the open field ($F_{2,42} = 4.97$, $p \leq 0.01$), with Arc^{-/-} mice being more active than Arc^{+/+} and Arc^{+/-} mice ($p < 0.008$; Figure 3B). Afterward, mice were injected with amphetamine (1.5 mg/kg), revealing a genotype \times time interaction effect ($F_{22,462} = 1.92$, $p < 0.008$). Locomotor activity responses to amphetamine were higher in Arc^{-/-} mice than in Arc^{+/-} and Arc^{+/+} mice ($p < 0.005$; Figure 3B). Thus, genetic mutations disrupting Arc produced a hyperactive phenotype and amphetamine supersensitivity consistent with rodents' correlates of schizophrenia-like symptoms. Importantly, these results may suggest a previously unexpected effect of Arc in the modulation of the dopaminergic system.

Arc Genetic Disruption Decreased Dopamine Turnover in the PFC and Increased Post-synaptic D2 Signaling in the Striatum

To investigate how Arc genetic mutations might affect the dopaminergic system, we first analyzed Arc mutants for dopamine content in PFC and striatum, two major areas involved in the dopamine

hypothesis of schizophrenia (Koch et al., 2014; Simpson et al., 2010; Weinstein et al., 2016; Winterer and Weinberger, 2004). In the PFC, an Arc effect was present on dopamine tissue content ($F_{2,71} = 6.72$, $p = 0.002$; Figure 4A), but not on noradrenaline (Figure S3). Arc^{-/-} mice had increased dopamine levels ($p = 0.003$) and a decrease in both the HVA:dopamine and DOPAC:dopamine ratios ($p < 0.05$; Figures 4B and 4C), suggesting a decreased dopamine turnover. In agreement, measurements of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, revealed that Arc knockout mice had reduced pTH(Ser40) levels ($F_{2,15} = 9.58$, $p < 0.003$; Figure 4D), a marker of TH enzymatic activity (Anzalone et al., 2012), and normal TH total protein levels ($p = 0.7$; Figure 4E). In contrast, in the striatum there was no Arc-dependent effect on the dopamine content ($F_{2,47} = 0.46$, $p = 0.63$; Figure S3) or on HVA and DOPAC ratios (Figure S3). These results indicate that Arc genetic disruption affects the maintenance of dopaminergic transmission in the PFC.

Alterations of the dopamine system in the PFC can change D2 receptor transmission in the striatum (Clarke et al., 2014; Pycock et al., 1980), and dopamine/D2 pathways in the PFC-striatal loop have been implicated in the manifestations of schizophrenia (Simpson et al., 2010; Winterer and Weinberger, 2004). Thus, we next analyzed total and surface protein levels of the D2 receptors in Arc mutants. Genetic reduction of Arc (both +/– and –/–) increased surface D2 in the striatum ($F_{2,6} = 228.41$, $p < 0.0001$, Figure 4F), but not in the PFC ($F_{2,6} = 1.98$, $p = 0.22$; Figure S4). In contrast, no Arc effect was evident in the total levels of D2 in the striatum ($F_{2,6} = 0.16$, $p = 0.85$; Figure 4G) and PFC ($F_{2,6} = 0.59$, $p = 0.58$; Figure S4). Several studies indicate that increased D2 transmission in the striatum is an important component in the pathophysiology of schizophrenia (Abi-Dargham et al., 2000; Laruelle, 1998; Simpson et al., 2010; Winterer and Weinberger, 2004; Kellendonk et al., 2006). In particular, D2 density and surface availability is increased in drug-naïve or drug-free patients with schizophrenia (Abi-Dargham et al., 2000; Laruelle, 1998).

To further examine striatal D2 signaling in Arc knockout mice, we analyzed total and phosphorylated levels of different dopamine/D2-related intracellular regulatory proteins in baseline conditions and following the amphetamine challenge used for behavioral experiments. The Ser40 pTH levels have been directly correlated with the activity of pre-synaptic D2 (Anzalone et al., 2012). Consistent with this, we found that amphetamine reduced striatal pTH levels ($F_{1,30} = 5.83$, $p < 0.02$), but in an Arc-independent manner ($F_{2,30} = 0.31$, $p = 0.74$; Figure 4I). The total levels of TH were unassociated with Arc ($F_{2,34} = 0.74$, $p = 0.49$), as well as with amphetamine treatment ($F_{1,34} = 0.13$, $p = 0.72$, Figure 4H). Thus, our data suggest that Arc might not alter presynaptic D2 pathways in the striatum.

Reduced expression of Akt kinase has been reported in patients with schizophrenia (Emamian et al., 2004; Zhao et al., 2006). We found a similar reduction of total Akt under basal condition in both Arc^{+/-} and Arc^{-/-} mice ($F_{2,32} = 8.97$, $p < 0.001$; Figure 4J). The thr308 pAkt levels have been directly correlated with the activity of postsynaptic D2 (Beaulieu et al., 2007). We found that at the basal level, Arc^{-/-} mice had reduced pAkt compared to Arc^{+/+} mice ($F_{2,29} = 4.77$, $p < 0.01$; Figure 4K). Following amphetamine treatment, Arc knockout mice did not show further

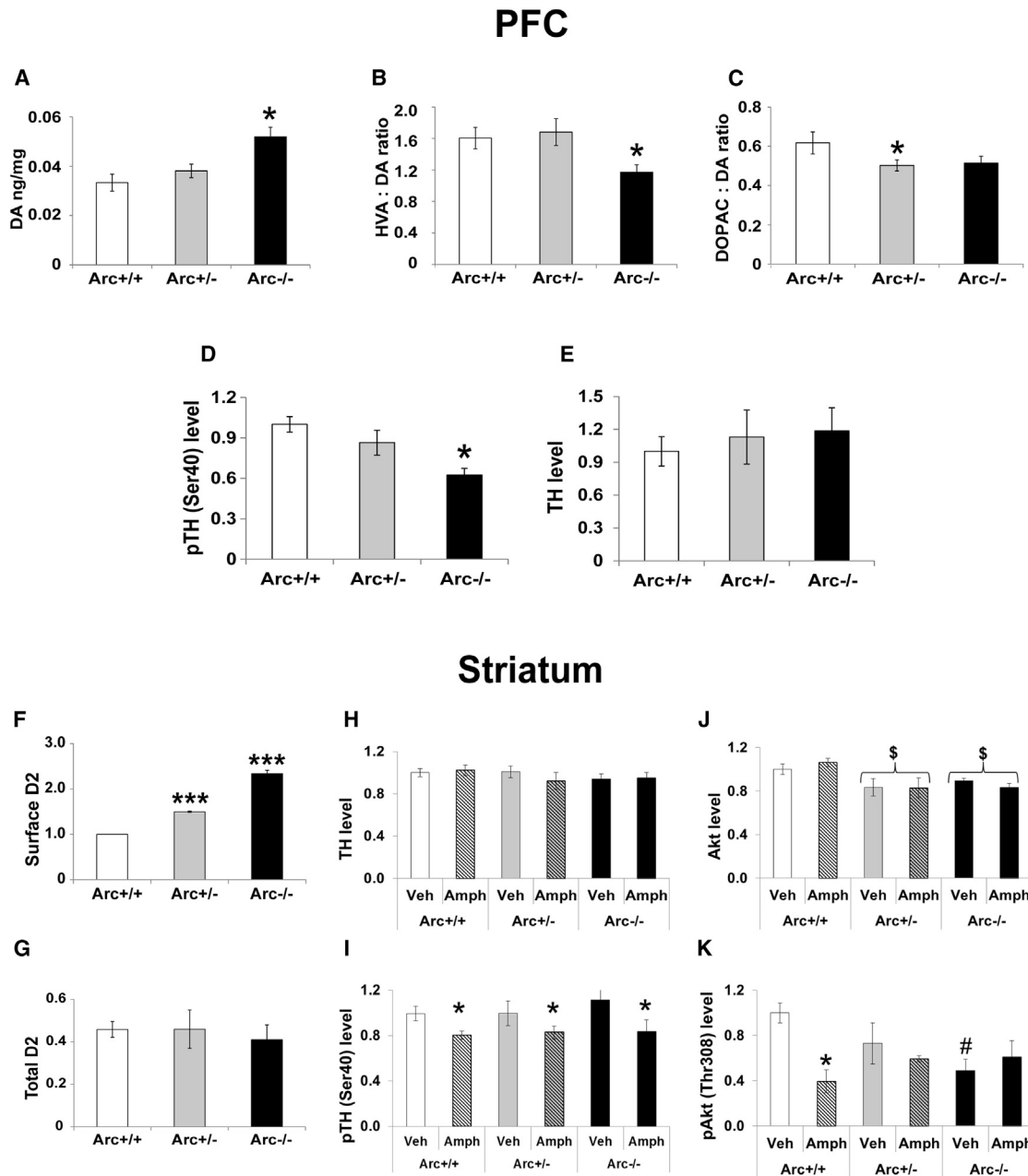


Figure 4. PFC Decreased Dopamine Turnover and Striatal Increased Post-synaptic D2 Signaling in Arc Knockout Mice

(A–E) Total dopamine (DA) content expressed as nanograms per milligram of tissue (A), HVA:DA (B) and DOPAC:DA (C) metabolite ratios, levels of TH phosphorylated at the Ser40 site (pTH Ser40) (D), and TH in dissected PFC (E) of Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates (n = 14–20/group for HPLC, n = 5–7/group for western blots). *p < 0.05 versus Arc^{+/+} mice.

(F–K) Densitometric analyses of D2 receptors (F and G) and relative levels of total TH (H), pTH (Ser40) (I), total Akt (J), and pAkt (Thr308) (K) in dissected striatum of Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates. For the D2 analysis, striatal samples were loaded 1:20 compared to PFC samples, and for each brain area, the input was 1:10 compared to the pull down. Samples without Streptavidin beads and Synaptophysin proteins were used as a pull-down control. Data are normalized to transferrin receptor proteins. For total TH and Akt levels, actin was used as loading controls, while for measurement of phospho-protein levels, total protein were used as a loading control. *p < 0.05 and ***p < 0.0005 versus Arc^{+/+}. #p < 0.05 versus Arc^{+/+} within the same treatment, \$p < 0.05 versus Arc^{+/+}. Representative western blots are shown in Figure S7.

reduction in pAkt, in contrast to wild-type mice. Taken together, the D2 expression and Akt data (Figures 4F–4K) suggest that Arc disruption increased postsynaptic dopamine/D2 signaling in the

striatum. Notably, mice with selective overexpression of post-synaptic D2 in the striatum show molecular and behavioral alterations (Kellendonk et al., 2006) overlapping with Arc knockout

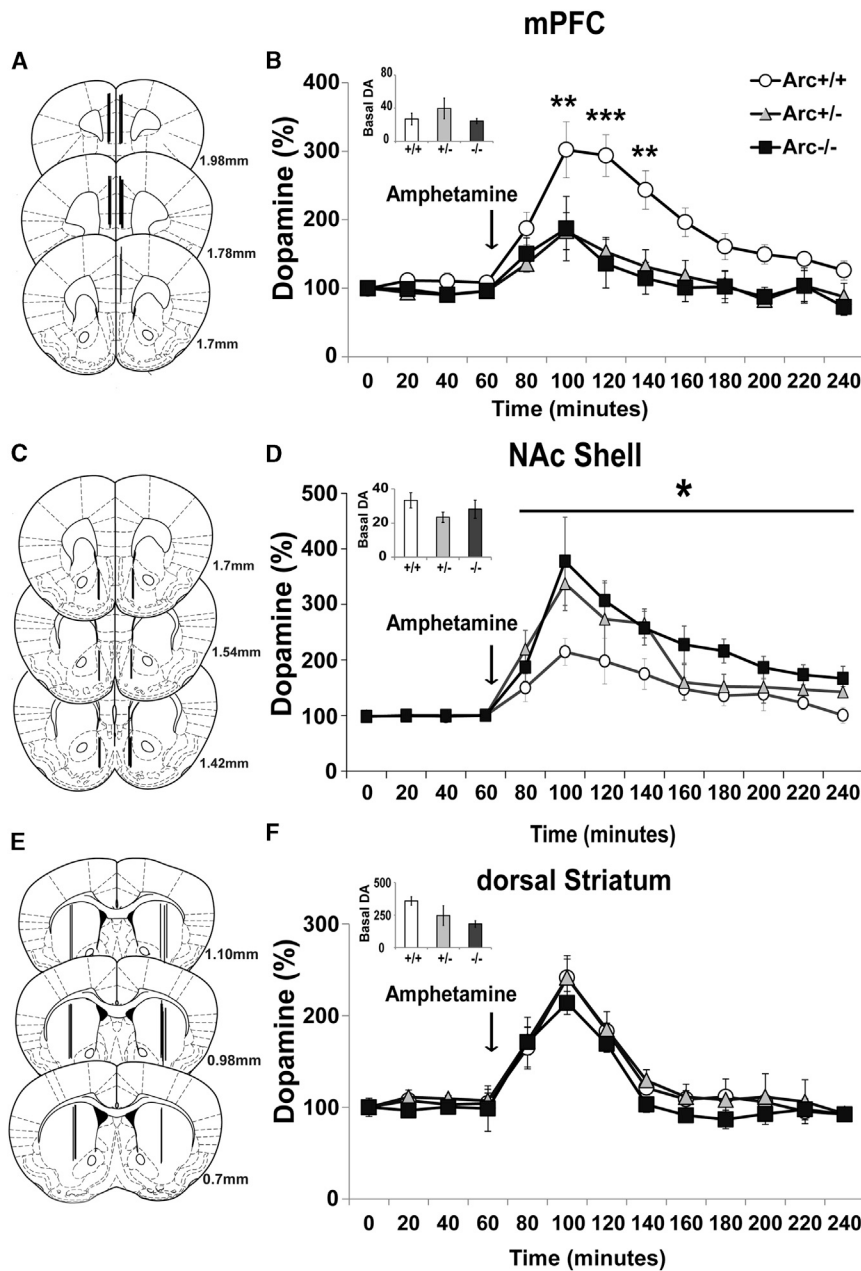


Figure 5. Opposite Regulation of Amphetamine-Evoked Dopamine Release in the mPFC and Nucleus Accumbens Shell in Freely Moving Arc Knockout Mice

(A, C, and E) Coronal sections showing the placements in mPFC (A), ventral striatum nucleus accumbens (NAc) shell portion (C), and dorsal striatum (E) of the in vivo microdialysis probes. (B, D, and F) Effect of amphetamine (1.5 mg/kg i.p.) on the dopamine (DA) dialysate levels in mPFC (B), nucleus accumbens shell (D), and dorsal striatum (F) in freely moving Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates.

Data are expressed as the percentage of change in dopamine extracellular levels from the basal values. Inset quadrants show basal values of extracellular dopamine expressed as fmol/20 μl sample. n = 6–8/group. ***p < 0.0005 and **p < 0.005 versus Arc^{+/-} and Arc^{-/-} within the same time point.

on dopamine release in the mPFC ($F_{2,20} = 6.1$, $p < 0.009$; Figures 5A and 5B), in the ventral striatum at the level of nucleus accumbens shell ($F_{2,11} = 4.31$, $p < 0.05$; Figures 5C and 5D), but not in the dorsal striatum ($F_{2,17} = 0.87$, $p = 0.44$; Figures 5E and 5F). In particular, compared to wild-type mice, amphetamine-dependent dopamine release in Arc knockout mice was reduced in the mPFC ($p < 0.02$; Figure 5B) but increased in the nucleus accumbens shell ($p < 0.05$; Figure 5D). We also replicated in these same mice the Arc-dependent locomotor supersensitivity to amphetamine (Figure S5). Consistently, previous studies indicated that pharmacological inhibition of PFC dopaminergic input leads to supersensitivity in amphetamine-induced locomotion (Tzschenke, 2001), and amphetamine-induced locomotion is preferentially related to an increase in dopamine neurotransmission in the nucleus accumbens shell more than to dorsal striatum (Ikemoto, 2002; Heidbreder and

mice, further supporting Arc as a converging point of schizophrenia genetics.

Arc Genetic Disruption Dampened Amphetamine-Induced Dopamine Release in the mPFC but Intensified It in the Nucleus Accumbens Shell

To directly and more precisely characterize the active processes of dopamine release, we measured its dynamics in the extrasynaptic space by in vivo microdialysis in freely moving Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates.

Notably, the same amphetamine challenge to which Arc knockout mice were supersensitive revealed a genotype effect

(Feldon, 1998). Together with the ex vivo measurements (Figure 4), these findings indicate that Arc genetic disruption results in a hypoactive PFC and a hyperactive striatal dopaminergic system. Importantly, these data parallel similar findings in patients with schizophrenia (Slifstein et al., 2015; Howes and Kapur, 2009).

Arc Genetic Disruption Reduced VTA-Induced Cortical Activity in the PFC

We next examined whether Arc PFC deficit may be associated with changes in frontal cortical activity. Electrophysiological studies have shown that dopaminergic input plays an important

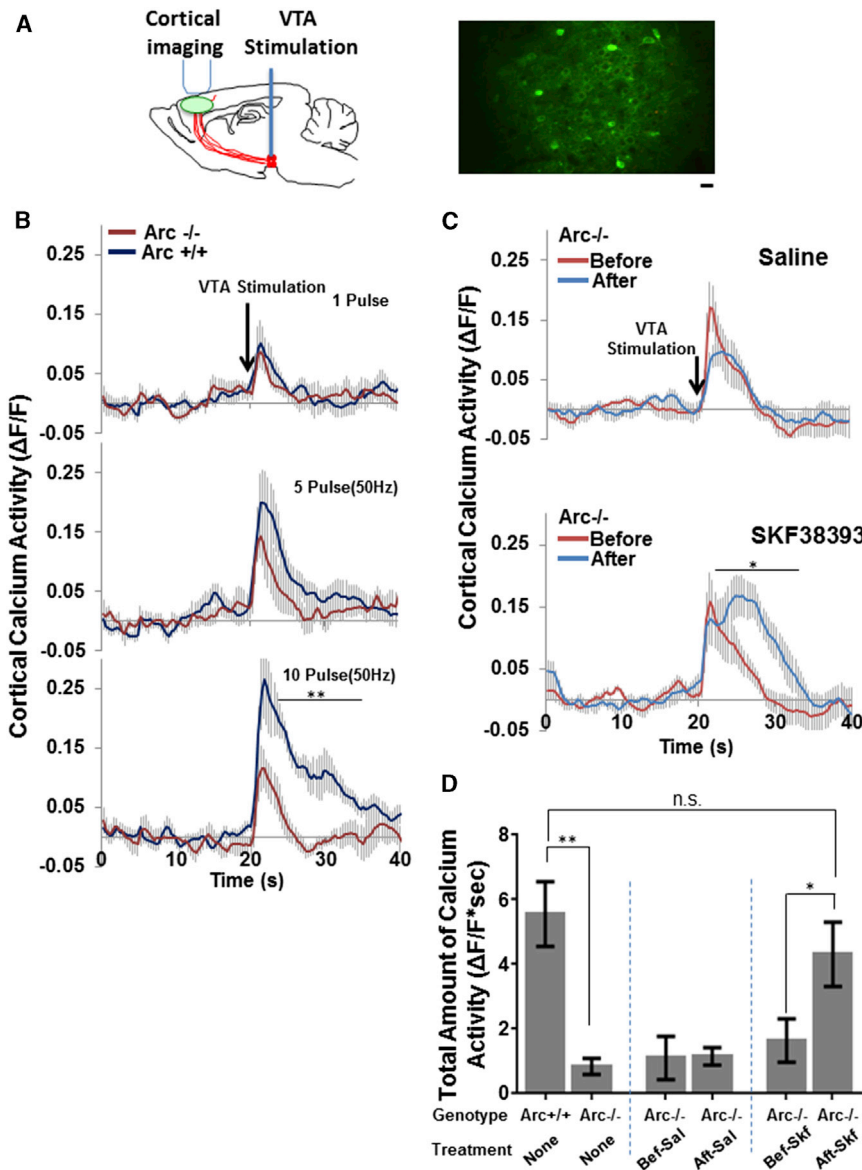


Figure 6. VTA-Induced PFC Activation Is Reduced in Arc Knockout Mice, and This Reduction Is Recovered by Pharmacological Activation of D1 Receptors

(A) Diagram showing the experimental setup for imaging VTA-stimulation evoked frontal cortical response (left). An AAV virus encoding a synapsin-promoter-driven calcium reporter GCaMP6 was locally injected into the PFC. GCaMP6 fluorescence was imaged in the superficial layers of the PFC by two-photon microscopy (right). Scale bar, 15 μ m.

(B) Time courses of cortical calcium signals in response to VTA stimulation in Arc^{+/+} and Arc^{-/-} mice. One, five, or ten pulses of electrical stimuli (50 Hz) were delivered at 20 s after the start of imaging. The cortical calcium activity at each time point is represented by the change in image fluorescence relative to the baseline image fluorescence ($\Delta F/F$). $n = 6/\text{group}$, ** $p < 0.001$ versus Arc^{+/+}.

(C) Time courses of VTA-stimulation evoked cortical responses in Arc^{-/-} mice before and after saline or D1 agonist SKF38393 injection (10 mg/kg, i.p.). Ten pulses of electrical stimuli (50 Hz) were delivered at 20 s after the start of imaging in each session. The frontal cortical activation was significantly increased after SKF38393 injection (* $p < 0.02$). $n = 6/\text{group}$.

(D) Total amount of calcium activity in the PFC following ten-pulse (50-Hz) VTA stimulation (sum of 20–35 s) showing significant reduction in Arc^{-/-} compared to Arc^{+/+} mice (** $p < 0.006$) and significant increase in Arc^{-/-} mice after SKF compared to before SKF (* $p < 0.02$). No significant difference between Arc^{+/+} and Skf-injected Arc^{-/-} animals ($p = 0.40$). $n = 6/\text{group}$.

role in regulating the activity of frontal cortical neurons (Robbins and Arnsten, 2009). For example, burst electrical stimulation of dopamine neurons in midbrain ventral tegmental area (VTA) evokes sustained neural activity in the frontal cortex (Lavin et al., 2005; Mastwal et al., 2014).

To efficiently sample the activity of neurons in the PFC and compare cortical activity between Arc wild-type and knockout mice, we adopted an in vivo optical imaging approach. We expressed the genetically encoded calcium sensor GCaMP6 in frontal cortical neurons through a locally injected adeno-associated virus (AAV) vector, and applied in vivo two-photon microscopy to image cortical calcium responses to VTA electrical stimulation (Figure 6A). While calcium activity cannot be equated with dopamine activation, we conducted experiments to test whether reduced dopamine release in the PFC of Arc knockout mice would lead to reduction in mesofrontal circuit activation.

to Arc^{+/+} animals (Figure 6B). This difference in activation becomes more prominent with increasing strength of VTA stimulation ($F_{1,10} = 20.94$, $p = 0.001$). Thus, our results show that the reduction of evoked cortical dopamine levels in Arc knockout mice is associated with reduced cortical response to VTA stimulation.

We next tested whether the impaired prefrontal response to VTA stimulation in Arc knock out mice could be rescued by pharmacologically enhancing dopaminergic signaling. Dopamine D1 receptors have been suggested to mediate the sustained neural activity of the frontal cortex in response to VTA activation (Lewis and O'Donnell, 2000). We confirmed that VTA evoked calcium responses in the PFC of wild-type mice were reduced by dopamine/D1 antagonist (Figure S6). Moreover, we administered D1 agonist SKF38393 (10 mg/kg, intraperitoneally [i.p.]) to Arc knockout mice and found that VTA-evoked frontal cortical

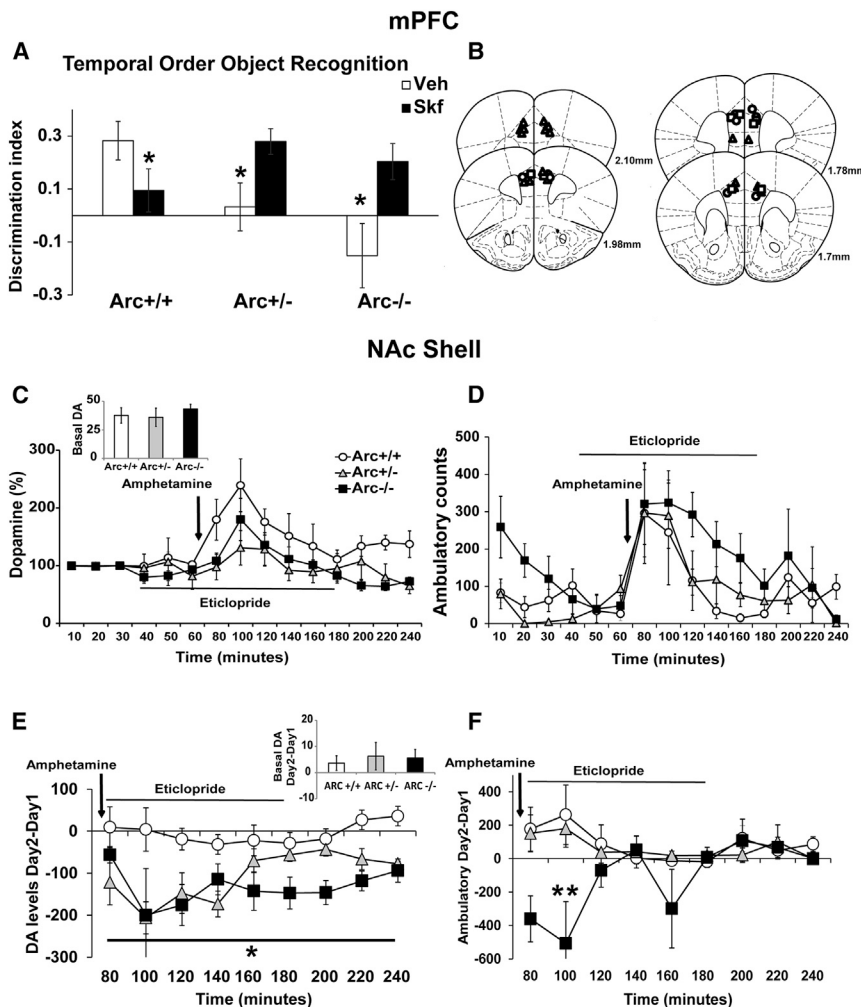


Figure 7. Pharmacological D1 Stimulation in mPFC and D2 Inhibition in the Nucleus Accumbens Shell Rescued Arc-Dependent Cognitive and Dopamine/Locomotor Deficits, Respectively

(A) Discrimination ratio displayed by Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates treated 15 min before the retrieval phase of the temporal order object recognition task with vehicle or the dopamine D1 agonist SKF38393 (0.06 μg in 0.3 μl), bilaterally microinjected in the mPFC (n = 5–15/group). *Groups showing no discrimination.

(B) Coronal sections showing the injection sites in mPFC for Arc^{+/+} (circles), Arc^{+/-} (triangles), and Arc^{-/-} (squares).

(C) Dopamine extracellular levels measured by in vivo microdialysis in the nucleus accumbens shell of freely moving Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} littermates in basal conditions, following the infusion of the D2-like antagonist eticlopride (1 μM) with reverse dialysis (30 min alone, and 2 hr after the i.p. injection of amphetamine 1.5 mg/kg) and during the 1-hr washout period. Data are expressed as the percentage of change in dopamine extracellular levels from the basal values. Basal values of extracellular dopamine (expressed as fmol/20-μl sample) are shown in the inset quadrants. n = 5–6/group.

(D) Ambulatory distance displayed by these same Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} mice while going through the different phases of the in vivo microdialysis experiment.

(E and F) Differences in dopamine levels (E), ambulatory activity (in beam crossing) (F), and basal dopamine levels (inset) between the day of eticlopride infusion and the day without eticlopride in Arc^{+/+}, Arc^{+/-}, and Arc^{-/-} mice. **p < 0.005 and *p < 0.05 versus Arc^{+/+}.

activation was significantly increased in contrast to saline control ($p < 0.027$, $F_{1,5} = 9.63$; Figure 6C). The total amount of calcium activity in the frontal cortex following ten-pulse (50 Hz) VTA stimulation was not different between Arc^{+/+} and SKF38393-injected Arc^{-/-} animals ($p = 0.397$; Figure 6D), indicating that the deficit in Arc^{-/-} mice is rescued by D1 agonist treatment. Taken together, these data support a deficiency of dopaminergic modulation in the PFC of Arc knockout mice and suggest that enhancing D1 signaling can rescue this circuit deficit.

D1 Stimulation in mPFC and D2 Inhibition in the Ventral Striatum Reverses Cognitive and Psychomotor Deficits, Respectively, in Arc Knockout Mice

The suggested dopaminergic imbalance in schizophrenia seems to involve a hypostimulation of D1 receptors in the PFC, as a possible mediator of negative symptoms and cognitive impairment, and a hyperstimulation of D2 receptors in striatal regions, as a possible mediator of positive symptoms (Slifstein et al., 2015; Tzschenke, 2001; Winterer and Weinberger, 2004). Thus, guided by our first findings (Figures 3, 4, 5, and 6), we directly tested this hypothesis in Arc knockout mice.

To rescue putatively PFC-dependent cognitive impairment in Arc mutant mice, we applied dopamine D1 agonist SKF38393 in the mPFC during the temporal order object recognition task. A genotype-by-treatment interaction effect was evident ($F_{2,47} = 3.87$, $p = 0.02$). In agreement with the in vivo two-photon optical imaging data (Figure 6), pre-test microinjection of the dopamine D1 agonist in the mPFC rescued the cognitive deficits found in Arc^{+/-} mice ($t = 5.36$; $df = 6$; $p = 0.002$; Figure 7A) and partially in Arc^{-/-} mice ($t = 2.57$; $df = 4$; $p = 0.08$; Figure 7A). In contrast, SKF38393 in the mPFC disrupted temporal order object recognition in wild-type mice ($t = 1.08$; $df = 6$; $p = 0.32$; Figure 7A). In mPFC vehicle-treated mice, we replicated the inability of Arc^{+/-} mice ($t = 0.35$; $df = 14$; $p = 0.73$) and Arc^{-/-} ($t = -1.14$; $df = 5$; $p = 0.31$) to distinguish between the object presented less recently and the one presented more recently (Figure 7A). These data are consistent with the inverted U-shaped relationship between cortical dopamine and cognitive performance (Papaleo et al., 2014; Vijayraghavan et al., 2007) and suggest that Arc genetic disruption generated dopamine/D1-mediated PFC deficiency and related cognitive deficits.

To rescue the psychomotor hyperactivity in Arc mutant mice, we applied D2-like antagonist eticlopride in the nucleus accumbens shell. The local infusion of eticlopride abolished the amphetamine-supersensitivity of Arc knockout mice, in terms of both dopamine release ($F_{2,55} = 1.70$, $p = 0.23$, [Figure 7C](#)) and locomotor responses ($F_{2,55} = 1.52$, $p = 0.26$, [Figure 7D](#)).

These data demonstrate that Arc knockout mice are more sensitive to a D2 antagonist. Compared to the phenotypes of these mice in the absence of eticlopride treatment, the D2 antagonism did not cause any significant change in $^{+/+}$ mice, but consistently reduced dopamine release ($F_{2,55} = 8.17$, $p = 0.007$; [Figure 7E](#)) and corresponding locomotor activation ($F_{2,55} = 5.56$, $p = 0.02$; [Figure 7F](#)) in Arc $^{+/-}$ and Arc $^{-/-}$ mice. Previous studies have indicated that elevated dopamine transmission in nucleus accumbens leads to hyperlocomotion ([Ikemoto, 2002](#)) and D2 antagonism in nucleus accumbens reduces psychostimulant-induced locomotion in a dose-dependent manner ([Baker et al., 1996](#); [Chausmer and Ettenberg, 1999](#); [van den Boss et al., 1988](#)). The stronger effect of eticlopride on amphetamine-induced locomotor activity in Arc mutant mice compared to Arc $^{+/+}$ mice is consistent with the elevated surface expression of D2 receptors in Arc mutant mice ([Figure 4F](#)). Taken together, these data suggest that the supersensitivity to amphetamine found in Arc knockout mice is mediated by altered D2 signaling in the striatum.

DISCUSSION

The data reported here show that genetic mutations resulting in loss or reduction in Arc function cause behavioral features spanning multiple domains of rodent correlates of cognitive, negative, and positive schizophrenia-like symptoms. Furthermore, our data implicate a previously unexplored role of Arc as a modulator of the brain dopaminergic system. The observed changes were consistent with a notion of schizophrenia pathophysiology that includes a hypodopamine/D1 functioning in the PFC but a hyperdopamine/D2 system in striatal regions. These findings provide initial biological evidence that genetic variations resulting in impaired Arc function contribute to pathophysiologic correlates of schizophrenia.

Arc knockout mice recapitulated many behavioral abnormalities considered rodent correlates of schizophrenia-like symptoms. In particular, Arc knockout deficits in PPI are analogous to PPI deficits found in patients with schizophrenia ([Swerdlow et al., 2008](#)). Similarly, the deficits in Arc knockout mice in sociability and social cognition are potentially mouse correlates of low social reciprocity and deficits in social cognition found in schizophrenia ([Millan et al., 2014](#)). Furthermore, Arc knockouts impairments in the temporal order and spatial object recognition tasks, with a preserved ability to recall and recognize objects in the novel object recognition task, is reminiscent to cognitive deficits found in schizophrenia. Indeed, patients with schizophrenia show analogous impairments in temporal and spatial memory tasks, while they are able to recall and recognize target items ([Dreher et al., 2001](#); [Schwartz et al., 1991](#); [Rizzo et al., 1996](#); [Folley et al., 2010](#)). Finally, Arc knockout mice's slight hyperactivity in a novel environment and amphetamine supersensitivity might parallel psychotic agitation and amphetamine's ability to be psy-

chogenic in healthy subjects and to exacerbates psychotic experiences in patients with schizophrenia ([van den Buuse, 2010](#)).

Earlier studies of Arc knockout mice have emphasized the deficits in long-term memory tasks such as the Morris water maze, auditory fear conditioning, and taste aversion, whereas the general performance and short-term memory of the knockout mice in these tasks were mostly normal ([Plath et al., 2006](#)). In this study, all the behavioral tasks were performed without prior training and do not require formation of long-term episodic memory. The deficits in these tasks therefore may reflect a pre-existing deficit in the steady-state function of the underlying brain circuits rather than the consolidation of new memories. Notably, the behavioral tasks reported in earlier studies mainly rely on the visual cortex, auditory cortex, taste cortex, hippocampus, or amygdala ([Plath et al., 2006](#)). By contrast, the tasks used in the current study involve PFC and also the dopaminergic system ([Barker et al., 2007](#); [Hanks et al., 2013](#); [Lacroix et al., 2000](#); [Tzschentke, 2001](#)). These findings raise the possibility that the different susceptibility of behavioral tasks to Arc genetic disruption may reflect differences in the underlying brain circuits. Furthermore, in the cortex and hippocampus, Arc is only expressed in the CamKII-positive excitatory neurons ([Ren et al., 2014](#); [Vazdarjanova et al., 2006](#)). Thus, Arc genetic disruption will not directly affect interneuron function in a cell-autonomous manner. However, as Arc genetic disruption leads to changes of both glutamate receptors in excitatory neurons and dopamine release, cortical inhibitory neuron activities will be affected through network interactions with dopamine and glutamatergic neurons. Some of the behaviors we assessed may indeed reflect this network level imbalance, as what has been proposed for the pathophysiology of schizophrenia.

More surprisingly, following Arc disruption, we identified contrasting deficits in the mesocortical and mesostriatal dopaminergic pathways. The Arc-dependent effects found in the PFC and in the ventral striatum, but not in the dorsal striatum, might be related to the fact that the source of dopamine in both the PFC and nucleus accumbens is the VTA, while in the dorsal striatum, it is the substantia nigra ([Beckstead et al., 1979](#)). The opposite effects of Arc genetics in amphetamine-induced dopamine release in PFC versus nucleus accumbens might originate from a circuit mechanism. Previous pharmacological studies have suggested that dopamine transmission in the PFC and nucleus accumbens are inversely coupled ([Scoraieni et al., 2009](#); [Simpson et al., 2010](#); [Tzschentke, 2001](#)) and direct anatomical connections with opposite feedbacks exist among the PFC, VTA, and nucleus accumbens to mediate these effects ([Carr and Sesack, 2000](#); [Ferenczi et al., 2016](#)). Remarkably, the Arc-dependent dopaminergic alterations resemble abnormalities considered key features in patients with schizophrenia (i.e., hypoactive D1 mesocortical and hyperactive D2 mesostriatal pathways) ([Simpson et al., 2010](#); [Slifstein et al., 2015](#); [Winterer and Weinberger, 2004](#)). Our findings suggest that Arc function is important for establishing the proper activity balance between mesocortical and mesostriatal dopaminergic circuits. Moreover, our studies further suggest that these circuit deficits can arise together from the same genetic background, which has been difficult to determine in humans due to different dopamine molecular imaging methods in striatal and cortical regions and genetic diversity ([Weinstein et al., 2016](#)).

Previous studies have reported that PFC dopaminergic inputs show protracted postnatal maturation through adolescence and are susceptible to activity-dependent modification during this period (Kalsbeek et al., 1988; Lewis and O'Donnell, 2000; Mastwal et al., 2014). Recurrent network activity in frontal-striatal loops can also affect striatal circuit maturation (Kozorovitskiy et al., 2012). As a neural activity-induced plasticity-related protein abundantly expressed in cortical excitatory and striatal GABAergic projection neurons (but not detected in midbrain dopamine neurons) (Shepherd and Bear, 2011), Arc may regulate activity-dependent maturation of the VTA-PFC-striatal circuits during postnatal development. Considering the well-known role of Arc in modulating glutamate receptors (Jakkamsetti et al., 2013; Ren et al., 2014; Shepherd and Bear, 2011), our findings raise the possibility that Arc-dependent changes in glutamatergic signaling might be the effector of the dopamine system changes. Since alterations in mesocortical and mesostriatal dopaminergic pathways are often inversely coupled to each other (Clarke et al., 2014; Simpson et al., 2010; Tzschenkte, 2001; Saunders et al., 1998), future studies are needed to differentiate the cell-autonomous and network effects of Arc genetic disruption.

In conclusion, Arc knockout mice recapitulate important features of rodent correlates of schizophrenia-related functional dimensions. Moreover, we describe a previously unexplored role of Arc in the modulation of dopaminergic neurotransmission, again consistent with schizophrenia-relevant endophenotypes. Together with the already well-known role of Arc in the modulation of the glutamatergic signaling (Ren et al., 2014; Jakkamsetti et al., 2013; Shepherd and Bear, 2011), the present data strongly implicate Arc as a key regulator of biological features associated with schizophrenia that involve PFC-striatal dopaminergic circuitry. Indeed, Arc may provide not only a converging point for genes encoding for glutamatergic post-synaptic proteins (Fromer et al., 2014) but also a crucial link between the dopaminergic and glutamatergic fronto-striatal systems.

EXPERIMENTAL PROCEDURES

Subjects

All procedures were approved by the Italian Ministry of Health (permit n. 230/2009-B) and local animal use committee and were in accordance with the *Guide for the Care and Use of Laboratory Animals* of the NIH and the European Community directives. Arc knockout mice (Wang et al., 2006) of both sexes (3–7 months old) were used. Because no sex-dependent differences were found in any parameter measured, male and female mice were pooled together. Arc null mutant (−/−) mice and their heterozygous (+/−) and wild-type (+/+) littermates were bred in-house by +/− mating. Mice were housed two to four per cage, in a climate-controlled animal facility (21 ± 2°C), and maintained on a 12-hr light/dark cycle (7 a.m. to 7 p.m.) with ad libitum access to food and water. Testing was conducted during the light phase. Different cohorts of mice were used for each task. Experimenters were blind to the genotype during testing.

Behavioral Experiments

Startle, PPI, and general health parameters were measured as previously described (Papaleo et al., 2008, 2012).

Locomotor activity, temporal order, spatial and novel object recognition tasks were performed as previously described (Huang et al., 2014; Papaleo et al., 2008; Barker et al., 2007).

Sociability and preference for social novelty task as previously described (Papaleo et al., 2011).

TOR with Skf Treatment

Mice were bilaterally implanted with 7-mm-long stainless steel guide cannulae (Unimed) under an isoflurane/oxygen mixture anesthesia. The mPFC stereotax coordinates used were antero posterior (AP) +1.95 mm, lateral (L) ±0.5 mm, and dorso ventral (DV) −1.5 mm, relative to the bregma (Franklin and Paxinos, 1997). Guide cannulae were secured in place with dental cement. Mice were then allowed to recover for 7 days after surgery. The Skf38393 D1 agonist (Sigma-Aldrich) was dissolved in 0.9% saline solution and then injected in a dose of 0.06 μg/0.3 μl/side. An injection needle was inserted into the guide cannula protruding 1 mm below (final DV, −2.5 mm), which was connected by plastic tubing to a 10-μl Hamilton syringe; the process took 1 min, with an additional 1 min to allow diffusion. The drug injection was performed 15 min before the temporal order object recognition task (TOR) testing phase. At the completion of the experiments, mice were sacrificed, and the brains were removed and fixed in a 4% paraformaldehyde solution. Cannula placements were determined by examining serial 80-μm coronal sections stained with cresyl violet.

HPLC

For dopamine determination by HPLC, PFC and striatum were rapidly dissected from naive Arc^{−/−}, Arc^{+/-}, and Arc^{+/+} littermates, immediately frozen in dry ice, and then stored at −80°C. Samples were then lysed in 0.1 M perchloric acid, then sonicated and spun in a microcentrifuge at 10,000 × g for 10 min. The supernatant was transferred in ultra-free microcentrifuge tubes (Millipore, catalog UFC30GV0S) and spun for 2 min. Samples (11 μl) were injected into the HPLC and measurements of dopamine and metabolites were made with an electrochemical detection system (ALEXYS LC-EC; Antec Leyden BV) equipped with a reverse-phase column (3-μm particles, ALB-215 C18, 1 × 150 mm; Antec Leyden BV) at a flow rate of 200 μl/min and electrochemically detected by a 0.7-mm glass carbon electrode (VT-03; Antec Leyden BV). The mobile phase contained 50 mM H₃PO₄, 50 mM citric acid, 8 mM KCl, 0.1 mM EDTA, 400 mg/l octanesulfonic acid sodium salt, and 3% (v/v) methanol (pH 7.5).

In Vivo Microdialysis

Vertical concentric dialysis probes, with a dialyzing portion of 2 mm for dorsal striatum and mPFC and 1 mm for nucleus accumbens shell, were prepared using AN69 fibers (Hospal Dasco), as previously described (De Luca et al., 2014). More details can be found in [Supplemental Experimental Procedures](#).

Electrical Stimulation Coupled with In Vivo Two-Photon Imaging Viral Injection

Using a glass micropipette connected to a 10-μl Hamilton syringe and a syringe pump, AAV1.Syn.Flex.GCaMP6s.WPRE.SV40 (1 μl) was injected into a single site in the PFC (from bregma: AP +2.0, medio lateral [ML] +0.5, and DV −0.3 mm) of Arc^{+/+} and Arc^{−/−} littermates. Animals were allowed to recover for ~1–2 weeks.

Electrode Implantation and Cranial Window

A bipolar stimulation electrode was lowered into the VTA (from bregma: AP −3.2 mm, ML 0.5 mm, and DV 4.5 mm) and glued in place in animals anesthetized with isoflurane (~1.5%). A cranial window was opened above the AAV-GCaMP6 injected region in the frontal cortex (from bregma: AP 1.0–3.0 mm, ML 0.3–1.3 mm, covering the M2/FrA region). The cranial window was filled with silicon gel, covered with a glass coverslip, and sealed with dental cement. A head plate was glued on the skull for fixation during imaging. The animals were then taken off the anesthesia and allowed to recover for ~1 hr before imaging.

VTA Stimulation and GCaMP6 Imaging

VTA was stimulated with electrical pulses (0.7 mA, 0.2 ms per pulse, and one, five, or ten pulses at 50 Hz per stimulus train). A two-photon microscope (FV1000, Olympus) was used to image the brain under the cranial window (excitation laser: 900 nm) using a 20× water-immersion lens (numerical aperture [NA] 0.95). Time series images lasting ~40 s (115 frames at 0.351 s/frame) were taken for each stimulus train, with the VTA stimulus delivered at 20 s after the start of imaging. For saline, SKF38393 (10 mg/kg, i.p.) and SCH23390 (1 mg/kg, i.p.) injection experiments, GCaMP6 response to the ten-pulse (50 Hz) VTA stimulation was imaged before and 30 min after the injection.

Image Analysis

Images were analyzed using NIH ImageJ. The mean pixel intensity in each image frame of the time series was calculated as Ft. Baseline fluorescence (F0) was defined as the average of the fluorescent signals (Ft) in the first 15 s of the time series. Changes in calcium signals ($\Delta F/F$) are calculated as $(F_t - F_0)/F_0$.

Slice Surface Biotinylation, Antibodies, and Western Blot Analyses

Details are reported in the [Supplemental Experimental Procedures](#).

Statistical Analysis

Results are expressed as mean \pm SEM throughout. One- or two-way ANOVAs were applied to compare the group means. Newman-Keul's post hoc test was used for making comparisons between groups when the overall ANOVA showed statistical significant differences for the main factors. The accepted value for significance was $p \leq 0.05$. Statistical analyses were performed using Statistica 11 (StatSoft).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Results, Supplemental Experimental Procedures, seven figures, and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2016.07.044>.

AUTHOR CONTRIBUTIONS

Conceptualization, F.M., D.R.W., K.H.W., and F.P.; Methodology, F.M., M.M., S.M., K.H.W., and F.P.; Investigation, F.M., M.M., S.M., D.S., M.E., R.M., M.D.L., and F.P.; Resource, K.H.W. and F.P.; Writing, F.M., D.R.W., K.H.W., and F.P.; Visualization and Analysis, F.M., S.M., and F.P.; Supervision, K.H.W. and F.P.; Funding Acquisition, D.R.W., K.H.W., and F.P.

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REFERENCES

Abi-Dargham, A., Rodenhiser, J., Printz, D., Zea-Ponce, Y., Gil, R., Kegeles, L.S., Weiss, R., Cooper, T.B., Mann, J.J., Van Heertum, R.L., et al. (2000). Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc. Natl. Acad. Sci. USA* *97*, 8104–8109.

Anzalone, A., Lizardi-Ortiz, J.E., Ramos, M., De Mei, C., Hopf, F.W., Iaccarino, C., Halbout, B., Jacobsen, J., Kinoshita, C., Welter, M., et al. (2012). Dual control of dopamine synthesis and release by presynaptic and postsynaptic dopamine D2 receptors. *J. Neurosci.* *32*, 9023–9034.

Baker, D.A., Khroyan, T.V., O'Dell, L.E., Fuchs, R.A., and Neisewander, J.L. (1996). Differential effects of intra-accumbens sulpiride on cocaine-induced locomotion and conditioned place preference. *J. Pharmacol. Exp. Ther.* *279*, 392–401.

Barker, G.R., and Warburton, E.C. (2011). When is the hippocampus involved in recognition memory? *J. Neurosci.* *31*, 10721–10731.

Barker, G.R., Bird, F., Alexander, V., and Warburton, E.C. (2007). Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *J. Neurosci.* *27*, 2948–2957.

Beaulieu, J.M., Gainetdinov, R.R., and Caron, M.G. (2007). The Akt-GSK-3 signaling cascade in the actions of dopamine. *Trends Pharmacol. Sci.* *28*, 166–172.

Beckstead, R.M., Domesick, V.B., and Nauta, W.J. (1979). Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res.* *175*, 191–217.

Cao, V.Y., Ye, Y., Mastwal, S., Ren, M., Coon, M., Liu, Q., Costa, R.M., and Wang, K.H. (2015). Motor Learning Consolidates Arc-Expressing Neuronal Ensembles in Secondary Motor Cortex. *Neuron* *86*, 1385–1392.

Carr, D.B., and Sesack, S.R. (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J. Neurosci.* *20*, 3864–3873.

Chausmer, A., and Ettenberg, A. (1999). Intraaccumbens raclopride attenuates amphetamine-induced locomotion, but fails to prevent the response-reinstating properties of food reinforcement. *Pharmacol. Biochem. Behav.* *62*, 299–305.

Chowdhury, S., Shepherd, J.D., Okuno, H., Lyford, G., Petralia, R.S., Plath, N., Kuhl, D., Haganir, R.L., and Worley, P.F. (2006). Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. *Neuron* *52*, 445–459.

Clarke, H.F., Cardinal, R.N., Rygula, R., Hong, Y.T., Fryer, T.D., Sawiak, S.J., Ferrari, V., Cockcroft, G., Aigbirhio, F.I., Robbins, T.W., and Roberts, A.C. (2014). Orbitofrontal dopamine depletion upregulates caudate dopamine and alters behavior via changes in reinforcement sensitivity. *J. Neurosci.* *34*, 7663–7676.

De Luca, M.A., Cauli, O., Morelli, M., and Simola, N. (2014). Elevation of striatal urate in experimental models of Parkinson's disease: a compensatory mechanism triggered by dopaminergic nigrostriatal degeneration? *J. Neurochem.* *131*, 284–289.

Dreher, J.C., Banquet, J.P., Allilaire, J.F., Paillère-Martinot, M.L., Dubois, B., and Burnod, Y. (2001). Temporal order and spatial memory in schizophrenia: a parametric study. *Schizophr. Res.* *51*, 137–147.

Dreher, J.C., Koch, P., Kohn, P., Apud, J., Weinberger, D.R., and Berman, K.F. (2012). Common and differential pathophysiological features accompany comparable cognitive impairments in medication-free patients with schizophrenia and in healthy aging subjects. *Biol. Psychiatry* *71*, 890–897.

Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M., and Gogos, J.A. (2004). Convergent evidence for impaired AKT1-GSK3 β signaling in schizophrenia. *Nat. Genet.* *36*, 131–137.

Ferenczi, E.A., Zalocusky, K.A., Liston, C., Grosenick, L., Warden, M.R., Amata, D., Katovich, K., Mehta, H., Patenaude, B., Ramakrishnan, C., et al. (2016). Prefrontal cortical regulation of brainwide circuit dynamics and reward-related behavior. *Science* *351*, aac9698.

Folley, B.S., Astur, R., Jagannathan, K., Calhoun, V.D., and Pearson, G.D. (2010). Anomalous neural circuit function in schizophrenia during a virtual Morris water task. *Neuroimage* *49*, 3373–3384.

Franklin, K.B.J., and Paxinos, G. (1997). *The Mouse Brain in Stereotaxic Coordinates* (Academic Press).

Fromer, M., Pocklington, A.J., Kavanagh, D.H., Williams, H.J., Dwyer, S., Gormley, P., Georgieva, L., Rees, E., Palta, P., Ruderfer, D.M., et al. (2014). De novo mutations in schizophrenia implicate synaptic networks. *Nature* *506*, 179–184.

Guillozet-Bongaerts, A.L., Hyde, T.M., Dalley, R.A., Hawrylycz, M.J., Henry, A., Hof, P.R., Hohmann, J., Jones, A.R., Kuan, C.L., Royall, J., et al. (2014). Altered gene expression in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Mol. Psychiatry* *19*, 478–485.

Hanks, A.N., Dlugolenski, K., Hughes, Z.A., Seymour, P.A., and Majchrzak, M.J. (2013). Pharmacological disruption of mouse social approach behavior: relevance to negative symptoms of schizophrenia. *Behav. Brain Res.* *252*, 405–414.

Heidbreder, C., and Feldon, J. (1998). Amphetamine-induced neurochemical and locomotor responses are expressed differentially across the

- anteroposterior axis of the core and shell subterritories of the nucleus accumbens. *Synapse* 29, 310–322.
- Howes, O.D., and Kapur, S. (2009). The dopamine hypothesis of schizophrenia: version III—the final common pathway. *Schizophr. Bull.* 35, 549–562.
- Hu, J., Sathanoori, M., Kochmar, S., Azage, M., Mann, S., Madan-Khetarpal, S., Goldstein, A., and Surti, U. (2015). A novel maternally inherited 8q24.3 and a rare paternally inherited 14q23.3 CNVs in a family with neurodevelopmental disorders. *Am. J. Med. Genet. A.* 167A, 1921–1926.
- Huang, H., Michetti, C., Busnelli, M., Managò, F., Sannino, S., Scheggia, D., Giancardo, L., Sona, D., Murino, V., Chini, B., et al. (2014). Chronic and acute intranasal oxytocin produce divergent social effects in mice. *Neuropsychopharmacology* 39, 1102–1114.
- Huentelman, M.J., Muppana, L., Corneveaux, J.J., Dinu, V., Pruzin, J.J., Reiman, R., Borish, C.N., De Both, M., Ahmed, A., Todorov, A., et al. (2015). Association of SNPs in EGR3 and ARC with schizophrenia supports a biological pathway for schizophrenia risk. *PLoS ONE* 10, e0135076.
- Ikemoto, S. (2002). Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D1/D2 agonists. *Neuroscience* 113, 939–955.
- Jakkamsetti, V., Tsai, N.P., Gross, C., Molinaro, G., Collins, K.A., Nicoletti, F., Wang, K.H., Osten, P., Bassell, G.J., Gibson, J.R., and Huber, K.M. (2013). Experience-induced Arc/Arg3.1 primes CA1 pyramidal neurons for metabotropic glutamate receptor-dependent long-term synaptic depression. *Neuron* 80, 72–79.
- Kalsbeek, A., Voorn, P., Buijs, R.M., Pool, C.W., and Uylings, H.B. (1988). Development of the dopaminergic innervation in the prefrontal cortex of the rat. *J. Comp. Neurol.* 269, 58–72.
- Kellendonk, C., Simpson, E.H., Polan, H.J., Malleret, G., Vronskaya, S., Winiger, V., Moore, H., and Kandel, E.R. (2006). Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. *Neuron* 49, 603–615.
- Koch, K., Rus, O.G., Reeß, T.J., Schachtzabel, C., Wagner, G., Schultz, C.C., Sorg, C., and Schlösser, R.G. (2014). Functional connectivity and grey matter volume of the striatum in schizophrenia. *Br. J. Psychiatry* 205, 204–213.
- Kozorovitskiy, Y., Saunders, A., Johnson, C.A., Lowell, B.B., and Sabatini, B.L. (2012). Recurrent network activity drives striatal synaptogenesis. *Nature* 485, 646–650.
- Lacroix, L., Broersen, L.M., Feldon, J., and Weiner, I. (2000). Effects of local infusions of dopaminergic drugs into the medial prefrontal cortex of rats on latent inhibition, prepulse inhibition and amphetamine induced activity. *Behav. Brain Res.* 107, 111–121.
- Laruelle, M. (1998). Imaging dopamine transmission in schizophrenia. A review and meta-analysis. *Q. J. Nucl. Med.* 42, 211–221.
- Laruelle, M., Abi-Dargham, A., Gil, R., Kegeles, L., and Innis, R. (1999). Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol. Psychiatry* 46, 56–72.
- Lavin, A., Nogueira, L., Lapish, C.C., Wightman, R.M., Phillips, P.E., and Seamans, J.K. (2005). Mesocortical dopamine neurons operate in distinct temporal domains using multimodal signaling. *J. Neurosci.* 25, 5013–5023.
- Lewis, B.L., and O'Donnell, P. (2000). Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D(1) dopamine receptors. *Cereb. Cortex* 10, 1168–1175.
- Mastwal, S., Ye, Y., Ren, M., Jimenez, D.V., Martinowich, K., Gerfen, C.R., and Wang, K.H. (2014). Phasic dopamine neuron activity elicits unique mesofrontal plasticity in adolescence. *J. Neurosci.* 34, 9484–9496.
- McCurry, C.L., Shepherd, J.D., Tropea, D., Wang, K.H., Bear, M.F., and Sur, M. (2010). Loss of Arc renders the visual cortex impervious to the effects of sensory experience or deprivation. *Nat. Neurosci.* 13, 450–457.
- Millan, M.J., Fone, K., Steckler, T., and Horan, W.P. (2014). Negative symptoms of schizophrenia: clinical characteristics, pathophysiological substrates, experimental models and prospects for improved treatment. *Eur. Neuropsychopharmacol.* 24, 645–692.
- Miyashita, T., Kubik, S., Lewandowski, G., and Guzowski, J.F. (2008). Networks of neurons, networks of genes: an integrated view of memory consolidation. *Neurobiol. Learn. Mem.* 89, 269–284.
- Papaleo, F., Crawley, J.N., Song, J., Lipska, B.K., Pickel, J., Weinberger, D.R., and Chen, J. (2008). Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice. *J. Neurosci.* 28, 8709–8723.
- Papaleo, F., Silverman, J.L., Aney, J., Tian, Q., Barkan, C.L., Chadman, K.K., and Crawley, J.N. (2011). Working memory deficits, increased anxiety-like traits, and seizure susceptibility in BDNF overexpressing mice. *Learn. Mem.* 18, 534–544.
- Papaleo, F., Yang, F., Garcia, S., Chen, J., Lu, B., Crawley, J.N., and Weinberger, D.R. (2012). Dysbindin-1 modulates prefrontal cortical activity and schizophrenia-like behaviors via dopamine/D2 pathways. *Mol. Psychiatry* 17, 85–98.
- Papaleo, F., Burdick, M.C., Callicott, J.H., and Weinberger, D.R. (2014). Epistatic interaction between COMT and DTNBP1 modulates prefrontal function in mice and in humans. *Mol. Psychiatry* 19, 311–316.
- Park, S., Park, J.M., Kim, S., Kim, J.A., Shepherd, J.D., Smith-Hicks, C.L., Chowdhury, S., Kaufmann, W., Kuhl, D., Ryzanov, A.G., et al. (2008). Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. *Neuron* 59, 70–83.
- Plath, N., Ohana, O., Dammermann, B., Errington, M.L., Schmitz, D., Gross, C., Mao, X., Engelsberg, A., Mahlke, C., Welzl, H., et al. (2006). Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. *Neuron* 52, 437–444.
- Purcell, S.M., Moran, J.L., Fromer, M., Ruderfer, D., Solovieff, N., Roussos, P., O'Dushlaine, C., Chambert, K., Bergen, S.E., Kähler, A., et al. (2014). A polygenic burden of rare disruptive mutations in schizophrenia. *Nature* 506, 185–190.
- Pycock, C.J., Kerwin, R.W., and Carter, C.J. (1980). Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. *Nature* 286, 74–76.
- Rasetti, R., Mattay, V.S., White, M.G., Sambataro, F., Podell, J.E., Zolnick, B., Chen, Q., Berman, K.F., Callicott, J.H., and Weinberger, D.R. (2014). Altered hippocampal-parahippocampal function during stimulus encoding: a potential indicator of genetic liability for schizophrenia. *JAMA Psychiatry* 71, 236–247.
- Ren, M., Cao, V., Ye, Y., Manji, H.K., and Wang, K.H. (2014). Arc regulates experience-dependent persistent firing patterns in frontal cortex. *J. Neurosci.* 34, 6583–6595.
- Rial Verde, E.M., Lee-Osbourne, J., Worley, P.F., Malinow, R., and Cline, H.T. (2006). Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor-mediated synaptic transmission. *Neuron* 52, 461–474.
- Rizzo, L., Danion, J.M., van der Linden, M., and Grangé, D. (1996). Patients with schizophrenia remember that an event has occurred, but not when. *Br. J. Psychiatry* 168, 427–431.
- Robbins, T.W., and Arnsten, A.F. (2009). The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. *Annu. Rev. Neurosci.* 32, 267–287.
- Saunders, R.C., Kolachana, B.S., Bachevalier, J., and Weinberger, D.R. (1998). Neonatal lesions of the medial temporal lobe disrupt prefrontal cortical regulation of striatal dopamine. *Nature* 393, 169–171.
- Schwartz, B.L., Deutsch, L.H., Cohen, C., Warden, D., and Deutsch, S.I. (1991). Memory for temporal order in schizophrenia. *Biol. Psychiatry* 29, 329–339.
- Scornaienko, R., Cantrup, R., Rushlow, W.J., and Rajakumar, N. (2009). Prefrontal cortical D1 dopamine receptors modulate subcortical D2 dopamine receptor-mediated stress responsiveness. *Int. J. Neuropsychopharmacol.* 12, 1195–1208.
- Shepherd, J.D., and Bear, M.F. (2011). New views of Arc, a master regulator of synaptic plasticity. *Nat. Neurosci.* 14, 279–284.
- Shepherd, J.D., Rumbaugh, G., Wu, J., Chowdhury, S., Plath, N., Kuhl, D., Huganir, R.L., and Worley, P.F. (2006). Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. *Neuron* 52, 475–484.

- Simpson, E.H., Kellendonk, C., and Kandel, E. (2010). A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. *Neuron* 65, 585–596.
- Slifstein, M., van de Giessen, E., Van Snellenberg, J., Thompson, J.L., Narendran, R., Gil, R., Hackett, E., Girgis, R., Ojeil, N., Moore, H., et al. (2015). Deficits in prefrontal cortical and extrastriatal dopamine release in schizophrenia: a positron emission tomographic functional magnetic resonance imaging study. *JAMA Psychiatry* 72, 316–324.
- Sullivan, P.F., Kendler, K.S., and Neale, M.C. (2003). Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch. Gen. Psychiatry* 60, 1187–1192.
- Swerdlow, N.R., Light, G.A., Cadenhead, K.S., Sprock, J., Hsieh, M.H., and Braff, D.L. (2006). Startle gating deficits in a large cohort of patients with schizophrenia: relationship to medications, symptoms, neurocognition, and level of function. *Arch. Gen. Psychiatry* 63, 1325–1335.
- Swerdlow, N.R., Weber, M., Qu, Y., Light, G.A., and Braff, D.L. (2008). Realistic expectations of prepulse inhibition in translational models for schizophrenia research. *Psychopharmacology (Berl.)* 199, 331–388.
- Tzschentke, T.M. (2001). Pharmacology and behavioral pharmacology of the mesocortical dopamine system. *Prog. Neurobiol.* 63, 241–320.
- van den Boss, R., Cools, A.R., and Ogren, S.O. (1988). Differential effects of the selective D2-antagonist raclopride in the nucleus accumbens of the rat on spontaneous and d-amphetamine-induced activity. *Psychopharmacology (Berl.)* 95, 447–451.
- van den Buuse, M. (2010). Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. *Schizophr. Bull.* 36, 246–270.
- Vazdarjanova, A., Ramirez-Amaya, V., Insel, N., Plummer, T.K., Rosi, S., Chowdhury, S., Mikhael, D., Worley, P.F., Guzowski, J.F., and Barnes, C.A. (2006). Spatial exploration induces ARC, a plasticity-related immediate-early gene, only in calcium/calmodulin-dependent protein kinase II-positive principal excitatory and inhibitory neurons of the rat forebrain. *J. Comp. Neurol.* 498, 317–329.
- Vijayraghavan, S., Wang, M., Birnbaum, S.G., Williams, G.V., and Arnsten, A.F. (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat. Neurosci.* 10, 376–384.
- Wang, K.H., Majewska, A., Schummers, J., Farley, B., Hu, C., Sur, M., and Tonegawa, S. (2006). In vivo two-photon imaging reveals a role of arc in enhancing orientation specificity in visual cortex. *Cell* 126, 389–402.
- Waung, M.W., Pfeiffer, B.E., Nosyreva, E.D., Ronesi, J.A., and Huber, K.M. (2008). Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. *Neuron* 59, 84–97.
- Weinstein, J.J., Chohan, M.O., Slifstein, M., Kegeles, L.S., Moore, H., and Abi-Dargham, A. (2016). Pathway-specific dopamine abnormalities in schizophrenia. *Biol. Psychiatry*, Published online March 13, 2016. <http://dx.doi.org/10.1016/j.biopsych.2016.03.2104>.
- Winterer, G., and Weinberger, D.R. (2004). Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. *Trends Neurosci.* 27, 683–690.
- Zhang, W., Wu, J., Ward, M.D., Yang, S., Chuang, Y.A., Xiao, M., Li, R., Leahy, D.J., and Worley, P.F. (2015). Structural basis of arc binding to synaptic proteins: implications for cognitive disease. *Neuron* 86, 490–500.
- Zhao, Z., Ksiazak-Reding, H., Riggio, S., Haroutunian, V., and Pasinetti, G.M. (2006). Insulin receptor deficits in schizophrenia and in cellular and animal models of insulin receptor dysfunction. *Schizophr. Res.* 84, 1–14.