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Do Astrocytes Respond to Dopamine?

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Abstract. Astrocytes are now recognised as important contributors to synaptic transmission control. Dopamine is a key neuromodulator in the mammalian brain and establishing the potential extent of its actions involving astrocytes is vital to our overall understanding of brain function. Astrocyte membranes can express receptors for dopamine, as well as dopamine transporters, but the full effects of dopamine on astrocytic physiology are still uncertain and its mode of action controversial. Here we overview the developing field of astrocyte-dopamine interaction, focusing on how dopamine affects the pre-eminent astrocytic intracellular signalling messenger – Ca^{2+} – and the available evidence for astrocyte-mediated effects of dopamine on neurons. We then discuss some of the methodological issues that need to be addressed to help move the field forward.

Introduction

The study of astrocytes has undergone a renaissance over the last three decades. With the advent of fluorescent Ca^{2+} imaging technology and increasingly advanced tools for genetic manipulation, astrocytes have revealed multifarious and dynamic intracellular Ca^{2+} activity (Rusakov et al., 2014, Volterra et al., 2014), as well as changes in other intracellular signalling pathways (see (Verkhratsky A, 2007) for detailed review), in response to common neurotransmitters (Shao and McCarthy, 1995, Cornell-Bell et al., 1990). Astrocytes are now considered important players in the machinery of the synapse – the 'tripartite synapse' – and much recent work has gone into how they can, in turn, modulate and control neuronal activity (Henneberger et al., 2010, Perea and Araque, 2007).

At this exciting time for astroglial research, it is unsurprising that increasing work is going into elucidating any involvement of astrocytes in the brain's dopaminergic system. The monoamine dopamine is a major modulatory neurotransmitter in the CNS, implicated in reward processing, decision-making and action initiation and termination (Iversen LL, 2010). Various disorders – Parkinson's disease, schizophrenia and ADHD amongst others – are hypothesised to be due, at least in part, to a malfunction of the dopaminergic system (for a detailed review see (Iversen LL, 2010, Nutt et al., 2015, Leyton and Vezina, 2014, Vaughan and Foster, 2013).

Astrocytes are widely reported to express dopaminergic receptors, and plasma membrane proteins capable of dopamine transport (Pelton et al., 1981, Vermeulen et al., 1994, Khan et al., 2001, Inazu et al., 2003). Activation of astrocytic dopamine receptors has been linked to changes in intracellular cyclic AMP (cAMP), free Ca^{2+} and αβcrystallin, amongst other signalling molecules (Zanassi et al., 1999, Shao et al., 2013, Parpura and Haydon, 2000), as has the metabolism of dopamine itself within the astrocyte soma after uptake. Cytoplasmic free Ca^{2+} is considered the pre-eminent astrocytic intracellular signalling molecule (Rusakov, 2015) and various studies have reported dopamine-induced astrocytic Ca^{2+} activity

(Parpura and Haydon, 2000, Requardt et al., 2012, Khan et al., 2001), but the precise nature of these signals, their molecular pathways and possible functions are still unclear.

In this review we detail and discuss evidence for astrocytic ability to sense dopamine, the reported effects of dopamine on astrocytic physiology and the ways in which astrocytes has been suggested to go on to affect local neuronal activity. We also consider the technical challenges in the field and how divergent methodology could account for some of the current controversies in the literature.

Astrocytes sense dopamine and respond in a multitude of ways

Dopamine receptors (DARs), first identified in neurons and more recently in astrocytes, are of a metabotropic type and five different receptor subtypes have been identified (D1, D2, D3, D4 and D5).

D1 –Type Receptors

D1 receptors (D1Rs) and D5 receptors (D5Rs) are excitatory in neurons – hence they have been classified together, as the 'D1-type receptors'– they appear to boost AMPA and NMDA receptor (Snyder et al., 1998, Flores-Hernandez et al., 2000) and L-type calcium-channel currents (Surmeier et al., 1995). Agonists binding to these receptors activate PKA via cyclic AMP (cAMP). cAMP is activated by adenyl cyclase (AC) via the g-protein αolf/αs subunit coupled to the intracellular domain of the receptor. There have been recent reports of a novel phosphatidylinositol (PI)-linked D1-like receptor (so called because of its high affinity to the selective D1-type receptor ligand SCH23390) in neurons and astrocytes, which couples to PLC through Gαq and can increase intracellular free Ca^{2+} (see below) (Liu et al., 2009, Ming et al., 2006). This receptor presents an intriguing way of stimulating the major Ca^{2+} signaling pathway in astrocytes through a molecular cascade traditionally associated with cAMP increase. Indeed, selective stimulation of this receptor through the agonist

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SKF83959 has been shown to trigger astrocytic calcium transients (Liu et al., 2009) in culture and concomitant ERK1/2 activation (Huang et al., 2012).

There is strong evidence from culture studies for expression of D1 and D5 receptors in rat astrocytes. From the striatum, autoradiographic dopamine receptor labelling has been reported (Hosli and Hosli, 1986), as has D1-type receptor mRNA expression (Zanassi et al., 1999, Miyazaki et al., 2004, Brito et al., 2004). Correspondingly, selective D1-type receptor stimulation with the specific agonists SKF38393 and SKF81297 has been found to raise intracellular astrocytic cAMP levels (Zanassi et al., 1999, Vermeulen et al., 1994, Requardt et al., 2010). Astrocytes cultured from the cortex also display cAMP rises in response to SKF38393, although they are not as pronounced (Zanassi et al., 1999). Cortical astrocytes stimulated with dopamine show D1/5R mediated intracellular NADH increase, via PKA, (Requardt et al., 2010) in situ and concomitant Ca^{2+} increases (Requardt et al., 2012) in vitro. 24h chronic stimulation with SKF39383 stimulates NGF and GDNF release from astrocytes (Ohta et al., 2010). See Figure 1 for a summary of proposed D1-type receptor mediated pathways.

Whereas the above studies come from rodent preparations, astrocytic D1-type receptors have also been reported in monkey and human cultured striatal astrocytes – with different receptor affinities compared to rat, indicating a possible species difference in astrocyte-dopamine interaction (Vermeulen et al., 1994).

D2 receptor

D2, D3 and D4 receptors are inhibitory in neurons – they inhibit AMPAR, NMDAR and L-type Ca^{2+} channel current (Iversen LL, 2010) – and are grouped together as 'D2-type receptors'. They can also oligomerize with D1Rs (Lee et al., 2004, Hasbi et al., 2010), CB1Rs (Kearn et al., 2005) and A2ARs (Torvinen et al., 2004, Torvinen et al., 2005). D2-like receptors inhibit AC activation via $\alpha i/o$ G-proteins and activate PLC via $G\beta\gamma$, increasing intracellular IP3 – an important excitatory pathway in astrocytes (Rusakov et al., 2011).

D2-type receptors have been localized on prefrontal cortical astrocytes in situ with electron microscopy in rat (Duffy et al., 2011), and human (Khan et al., 2001) samples, and D2-type receptor RNA has been found in rodent striatal astrocytes (Bal et al., 1994, Miyazaki et al., 2004). The D2 receptor agonist ropinerole (applied over varying time periods) stimulates NGF and GDNF secretion from cultured rat cortical astrocytes (Ohta et al., 2010) and selective D2R activation with quinpirole inhibits αcrystallin-mediated inflammation-associated astrogliosis in the prefrontal cortex of rats in vivo (Shao et al., 2013). In rat hippocampal astrocytes in situ, D2-like receptors mediate apomorphine (a non-specific dopamine receptor agonist) induced S100B secretion (Nardin et al., 2011). In cultured rodent prefrontal cortical astrocytes, the D2 agonist quinpirole has been reported to marginally increase Ca^{2+} (see below) (Khan et al., 2001). See Figure 1 for a summary of proposed D2-type receptor mediated pathways.

Dopamine stimulation triggers protein tyrosine phosphorylation in cultured human glioma cells in a D2R-dependent manner (Luo et al., 1999), D2Rs are also upregulated in glioma cells and have been shown to be pro-proliferative (Li et al., 2014).

Non-specific DAR activation

Many studies have recorded changes in astrocytic activity in response to non-specific dopaminergic stimulation with both exogenously applied agonists and endogenously (Parpura and Haydon, 2000, Reuss and Unsicker, 2001, Li et al., 2006). Striatal astrocytic FGF-2 expression in response to apomorphine has been reported to be both D1 and D2 receptor dependent (Li et al., 2006). It is interesting to note that functional hetero-oligomerization of D1 and D2 receptors has been found in cells co-expressing both receptor types in striatal neurons of the rat brain (Hasbi et al., 2010) – activation of this hetero-oligomer increases intracellular Ca^{2+} (in HEK cells) in a PLC- dependent fashion (Lee et al., 2004), via the Gq/11 protein (Rashid et al., 2007). Given the importance of the Ca^{2+} signaling pathway in astrocytes, it would be interesting to see if this hetero-oligomer mediated dopaminergic signaling in astrocytes.

A recent study has also found that stimulation of cultured cortical rat astrocytes with dopamine leads to an increase in TNFα secretion via toll-like receptor 4 (TLR4) activation (Ding et al., 2015) – raising the prospect of an entirely new, DAR-insensitive form of astrocytic dopaminergic signaling. Dopaminergic modulation (after chronic application of dopamine) of astrocytic Ca^{2+} response to NMDA challenge has also been reported (Ding et al., 2014) in rodent cortical astrocyte cultures, as has astrocytic GLT-1 upregulation in response to dopaminergic fibre dennervation in the rodent prefrontal cortex (Vollbrecht et al., 2014).

Dopamine uptake and metabolism

Dopamine is transported from the extracellular space across the plasma membrane into the cytosol via uptake1 system and uptake2 system (Koepsell et al., 2007). Uptake1 is faster, higher-affinity, presynaptic neuron-based transport whereas uptake2 is slower, lower-affinity, extraneuronal transport.

Uptake1 occurs primarily through the dopamine transporter (DAT), although the norepinephrine transporter (NET) can also transport dopamine (indeed the NET has greater affinity for dopamine than norepinephrine (Pacholczyk et al., 1991)). Both are a part of the family of Na^+/Cl^- dependent neurotransmitter transporters and transport two Na^+ ions and one Cl^- ion into the cytosol with each dopamine molecule (Torres et al., 2003), down the Na^+ electrochemical gradient.

Classically the DAT and NET are considered to be only expressed on presynaptic neurons in situ (Lorang et al., 1994, Ciliax et al., 1995, Schroeter et al., 2000), but there is accumulating evidence that cultured astrocytes also express dopamine transport machinery (Pelton et al.,

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1981, Inazu et al., 1999a, Takeda et al., 2002, Schroeter et al., 2000).

Cultured astrocytes, taken from whole rat brain, express rapid Na$^+$-dependent dopamine uptake1 machinery (Pelton et al., 1981). It has been suggested that astrocytic DA uptake1 is mediated not by DAT, but by NET: DAT-specific blockers inhibit astrocytic dopamine uptake far less than NET-specific ones (Takeda et al., 2002, Inazu et al., 1999a). Evidence for NET expression in astrocytes comes from radioactive tracer uptake studies in cultured rat neocortical astrocytes (Inazu et al., 1999a, Takeda et al., 2002, Inazu et al., 2003) and mRNA expression in cultured rat spinal astrocytes (Schroeter et al., 2000). There are even conflicting reports over whether astrocytes even express DAT (Takeda et al., 2002) or not (Kittel-Schneider et al., 2012).

Uptake2 is mediated by organic cation transporter (OCT) and plasma membrane monoamine transporter (PMAT), independently of Na$^+$ and Cl$^-$ and is recognised to play an important role in dopamine clearance from the synapse (Baganz et al., 2008, Bacq et al., 2012, Ordway GA, 2007). Human astroglioma cells are reported to transport dopamine via uptake2 in a Na$^+$-independent manner (Russ et al., 1996), through OCT3 and PMAT – for which they express the mRNA (Naganuma et al., 2014).

So far, there is currently no consistent in situ evidence to support normal astrocytic expression of either DAT or NET. The number of studies reporting DAT or NET transport in astrocytic culture says as much about the remarkable plasticity of astrocytes as it does about their involvement in dopamine transport. Although it is clear astrocytes can selectively express dopamine uptake mechanisms (expression which is itself plastic through FGF and EGF (Inazu et al., 1999b)) and that DAT can be expressed in pathologically stimulated astrocytes in vivo – after treatment with L-DOPA in 6-OHDA parkinsonian model rats (Asanuma et al., 2014) – whether they do under normal conditions in an intact brain remains to be ascertained.

Astrocytes are able to metabolise dopamine intracellularly, as they express both catechol-O-methyl transferase (COMT) and mono-amine oxidase (MAO) (Pelton et al., 1981, Hansson and Sellstrom, 1983). COMT catalyzes the transfer of a methyl group from S-adenosyl-methionine to a hydroxyl group on the catechol nucleus of dopamine (Chen et al., 2004). MAO deaminates dopamine – a reaction that produces H2O2 via FAD (Youdim and Bakhle, 2006).

The H2O2 produced by the breakdown of dopamine by MAO has been linked to an increase in rat cortical astrocyte intracellular calcium (Vaarmann et al., 2010) in culture. H2O2 activates lipid peroxidation which activates PLC, causing the release of IP3 which increases the likelihood of Ca^{2+} release from intracellular stores (Vaarmann et al., 2010) (Figure 2A).

Antipsychotics

The pathogenesis of schizophrenia has been linked to changes in astrocytes (Schnieder and Dwork, 2011).
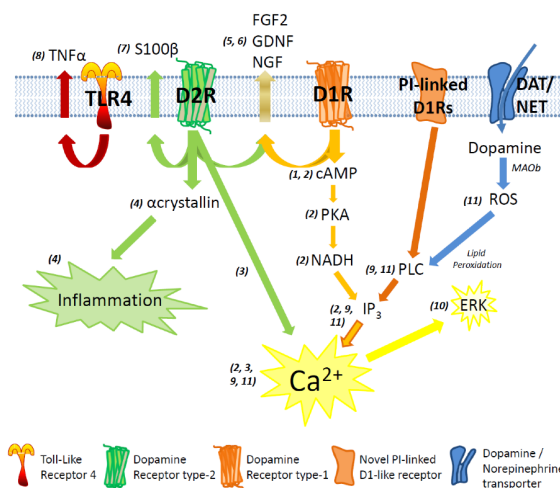

Figure 1. Putative pathways for dopaminergic signalling in astrocytes. D1-type receptor (D1R) stimulation increases cytoplasmic cyclic AMP (cAMP), leading to an increase in Protein Kinase A (PKA), NADH, IP3 and Ca^{2+}. D2-type receptor (D2R) stimulation leads to an increase in Ca^{2+}, S100β secretion, and also inflammatory response mediated via αcrystallin. Joint activation of D1Rs and D2Rs causes secretion of Fibroblast Growth Factor 2 (FGF2), Glial-Derived Neurotrophic Factor (GDNF) and Nerve-Growth Factor (NGF). Activation of novel phosphatidyl-inositol-linked D1R stimulates intracellular Ca^{2+} rise via PLC and IP3 increase. Metabolism of dopamine via Monoamine Oxidase B (MAOb) creates Reactive Oxygen Species (ROS) and activates the PLC/IP3/Ca^{2+} pathway. Ca^{2+} increase triggered through novel PI-linked D1R leads to ERK expression.

Antipsychotics used to treat schizophrenia, are known to act through blockade of D2-type receptors (Carlsson and Lindqvist, 1963) – through which they exert some of their antipsychotic effects (Meltzer and McGurk, 1999). Clozapine, an atypical neuroleptic, is one such drug – it attenuates schizophrenia-induced GFAP expression in astrocytes in a rat model of schizophrenia (Gomes et al., 2015).

Interestingly, clozapine appears to directly affect astrocytes in addition to any effects it has by blocking DARs. After clozapine incubation, cultured rat astrocytes (mixed cortex and striatum) show decreased Ca^{2+} response to dopamine challenge (Reuss and Unsicker, 2001) and enhanced d-serine and l-glutamate release (Tanahashi et al., 2012). Cultured glioma cells show enhanced S100β release after clozapine incubation (Steiner et al., 2010), and clozapine inhibits proteomic change in MK801-treated (a pharmacological model of schizophrenic physiology) glioma cells in culture (Martins-de-Souza et al., 2011).This clozapine-mediated astrocytic activity is very interesting, but so far no possible mechanism of action has been proposed for clozapine's effects on astrocytes. It presents another interesting locus of interaction between astrocytes and dopamine – one that merits further study.

Astrocytic calcium signalling and dopamine

Much work has gone into interrogating astrocytic Ca^{2+} signalling, given the complicated intracellular astrocytic Ca^{2+} dynamics witnessed in vitro (Cornell-Bell et al., 1990), in situ (Di Castro et al., 2011) and in vivo (Gourine et al., 2010), in response to neurotransmitter simulation (Shao

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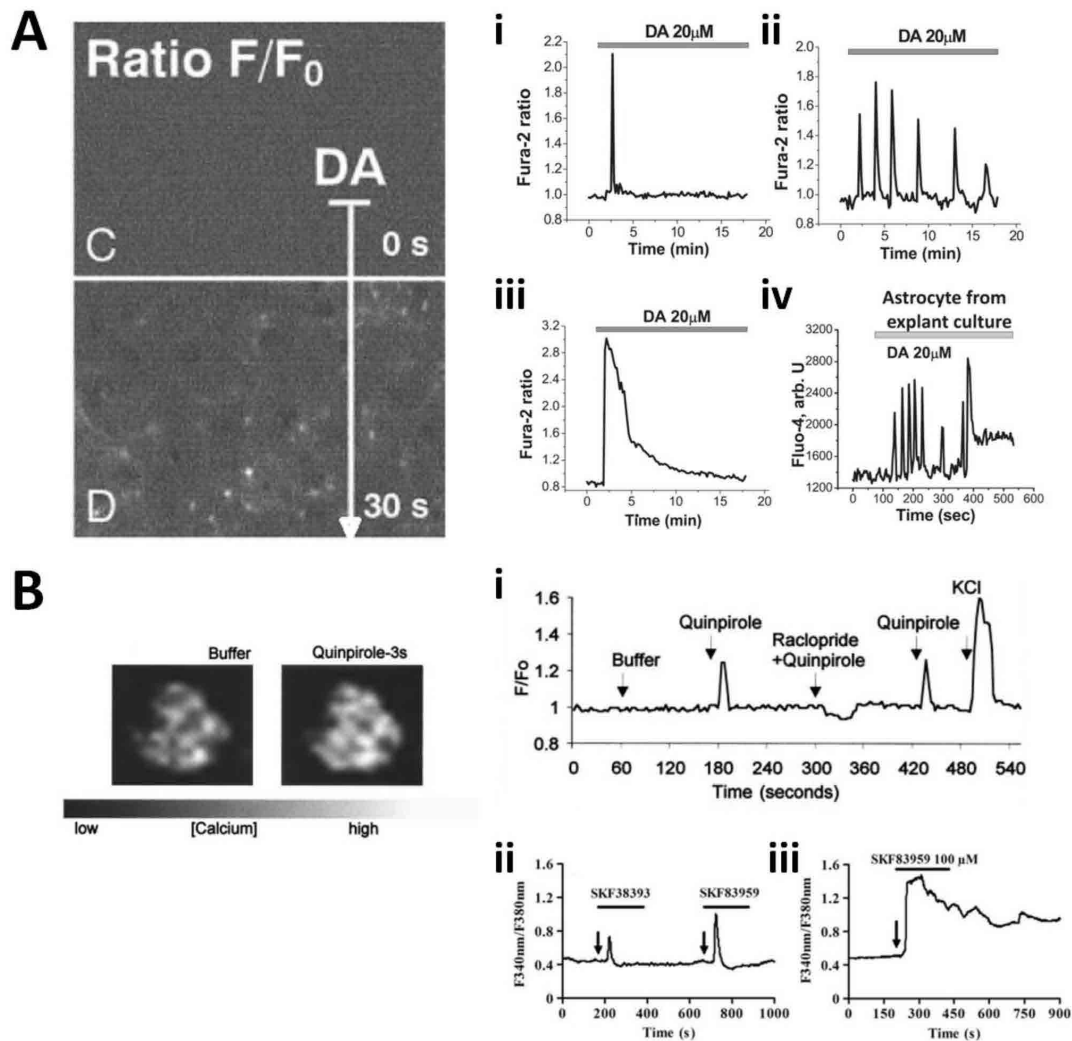
Figure 2. Dopamine-induced astroglial Ca^{2+} signals. A. Picture: cultured rodent cortical/striatal astrocytes display Ca^{2+} increases – measured with fluo-3 AM – fluorescence upon challenge with 100μM dopamine (adapted from Reuss and Unsicker, 2001). A i-iii. Example traces of differing Ca^{2+} responses – measured with Fura-2 – to an identical stimulus, 20μM dopamine, in primary cultured cortical astrocytes. A iv. Cortical astrocytes from explant cultures display different Ca^{2+} activity – measured with fluo-4 AM (adapted from Vaarmann et al. 2010). B. Picture: cultured rodent cortical astrocytes respond to 150μM of the D2R agonist quinpirole with an increase in Ca^{2+} - measured with fluo-3 – in left panel, compared to baseline in right panel. B i. Example trace of cortical astrocyte response to 150μM quinpirole (adapted from Kahn et al. 2001). B ii, iii. Stimulation of rodent cortical astrocyte Ca^{2+} - measured with fura-2 AM – with the novel PI-linked D1-like receptor agonist SKF83959 at 25μM (ii) and 100μM (iii) (adapted from Liu et al. 2009).

and McCarthy, 1995), neuronal activity (Navarrete and Araque, 2010) and physiological stimuli (Gourine et al., 2010). Astrocytes also display spontaneous Ca^{2+} activity that can be modulated (Di Castro et al., 2011, Requardt et al., 2012). Astrocytic Ca^{2+} signalling can trigger release of neuroactive and glioactive gliotransmitters from astrocytes (Perea and Araque, 2007, Gourine et al., 2010), through exocytosis (Perea and Araque, 2007) and hemichannel opening (Montero and Orellana, 2015), and the debate still rages as to the extent of their involvement in information processing in the brain (Rusakov et al., 2011). Hence, astrocytic Ca^{2+} response to dopamine is of particular interest; however until now the problem has only been addressed using experiments in vitro. Rodent cortical astrocytes in culture are sensitive to concentrations of dopamine above 5μM, and respond through single sharp Ca^{2+} single transients, repeated Ca^{2+} transients or broader Ca^{2+} increases (Figure 2A) (Vaarmann et al., 2010,

Parpura and Haydon, 2000, Reuss and Unsicker, 2001). The amplitude and frequency of these Ca^{2+} transients increases with dopamine concentration (Requardt et al., 2012) and have been reported to reach peak concentrations of 400 to 2500nM (Vaarmann et al., 2010, Parpura and Haydon, 2000). It has not been possible it appears to predict or control the precise kind of Ca^{2+} activity that dopamine application will induce in cultured astrocytes (Figure 2A) (unlike, say, action potential shape triggered by a constant depolarization in neurons). However, inhibition of dopamine-induced Ca^{2+} elevations has been reported after blockade of MAOb (Vaarmann et al., 2010), or following suppression of D1Rs and NADH activity (Requardt et al., 2012). Slight dopamine-induced Ca^{2+} increases (~20% above baseline) had previously been reported following D2R stimulation in rodent cortical astrocytes (Khan et al., 2001) (Figure 2Bi), as had dose-dependent Ca^{2+} increases following selective D1-like

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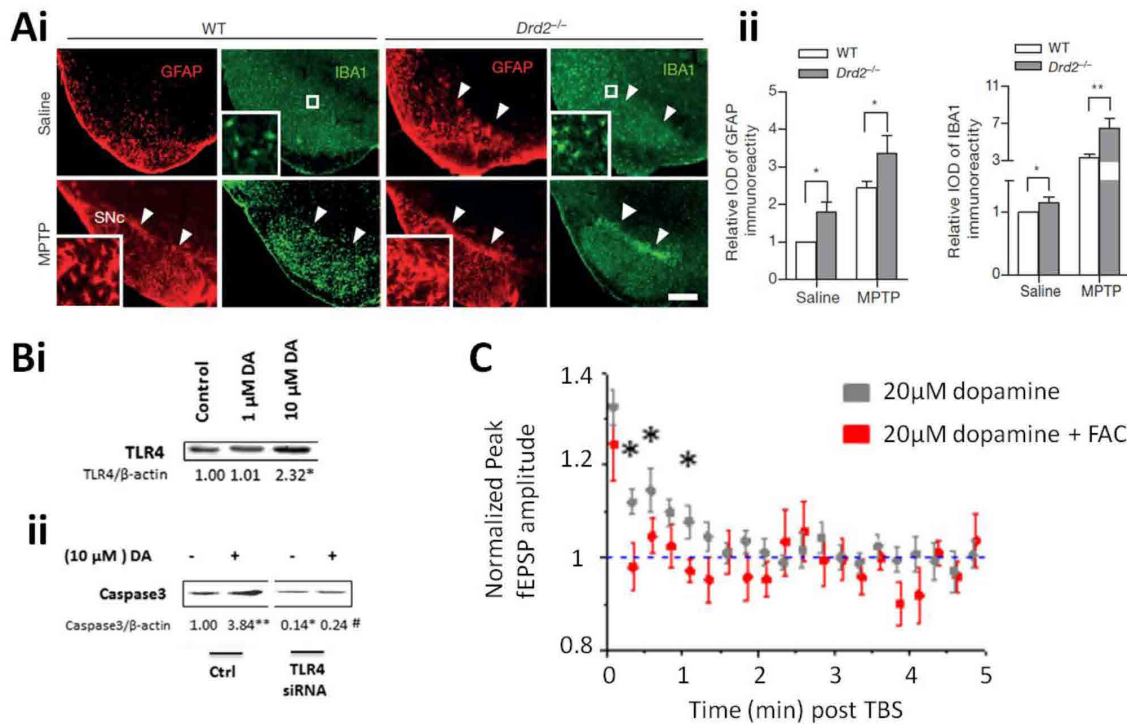
Figure 3. Dopaminergic effects on neurons through astrocytes. A i. DRD2-null mice – left panel – show greater inflammatory response, as measured by GFAP and IBA1 expression, in midbrain astrocytes in avivo after both saline and MPTP injections. A ii. Summary of data from (Ai) (adapted from Shao et al. 2013). B i. Cortical astrocytes co-cultured with neurons express increased TLR4 upon stimulation with 10μM dopamine. B ii. Neurons co-cultured with cortical astrocytes stimulated with 10μM dopamine express caspase3, but do not when astrocytic TLR4 has been knocked down with siRNA (adapted from Ding et al. 2015). C. Post-tetanic potentiation in the Stratum Lacunosum-Moleculare in the presence of 20μM dopamine is attenuated after pre-incubation with the glial poison fluoroacetate (FAC) (adapted from Jennings, 2014).

receptor stimulation in rat cortical astrocytes (Liu et al., 2009) (Figure 2Bii, iii). Although none of the above studies agree on the overall nature of the DA signalling pathway, they consistently reported that astrocytic dopaminergic Ca²⁺ signals are mediated by IP3 release into the cytosol – a well-documented astrocyte Ca²⁺ signalling pathway.

Astrocyte-mediated effects of dopamine on neurons

An apparent link between dopamine-induced Ca²⁺ elevation and Ca²⁺-dependent gliotransmitter exocytosis, GDNF, NGF, TNFα and inflammatory response (Parpura and Haydon, 2000, Ohta et al., 2010, Shao et al., 2013, Ding et al., 2015) raises the exciting theoretical possibility of dopamine-induced astrocytic communication with neurons . However, only two recent studies have shown this in a relatively direct manner. Shao et al. (2013) found that astrocytic DRD2 null mice expressed increased markers for astrocytic inflammatory activation (Figure 3A), which in turn lead to increased vulnerability of neurons to stress-induced apoptosis in the midbrain in vivo. They suggest that tonic activation of astrocytic DRD2s in the healthy brain regulates immune response via αβ-crystallin – a previously unreported signalling pathway. Ding et al. (2015) find that dopamine hyperstimulation (10μM for 24h) of neuron/astrocyte co-cultures triggers neuronal cell death mediated by astrocytic release of TNFα via dopaminergic stimulation of TLR4 (Figure 3B).

Astrocytes are known to release TNFα in both healthy and pathological situations (Santello and Volterra, 2012), but this dopamine/TLR4 pathway represents a novel mode of TNFα stimulation. Interestingly, TNFα is one of the few inflammatory pathways not upregulated in DRD2 null mice (Shao et al., 2013) – adding weight to the idea that TNFα secretion is under control of TLR4.

So far, the only direct evidence in the literature of a dopaminergic effect on neurons via astrocytes comes from studies of astrocyte-mediated neurotoxicity (Shao et al., 2013, Ding et al., 2015). There is no literature directly examining any putative dopaminergic regulation of normal brain function, such as synaptic plasticity or homeostasis – although astrocytes are thought to be integral to these processes (Rusakov et al., 2014, Henneberger et al., 2010). In our recent attempt to detect such phenomena (Jennings, 2014) we examined putative astrocytic involvement in the dopaminergic inhibition of post-tetanic potentiation (PTP) at the perforant-path – CA1 synapse in the Stratum Lacunosum-Moleculare in the hippocampus, the prominent inhibition phenomenon reported earlier (Otmakhova and Lisman, 1999) . The effects were not conspicuous: PTP was even more attenuated in the presence of dopamine (20μM) after glial poisoning with fluoroacetate (FAC) (Figure 3C). Accurate interpretation of such results could be complicated, partly down to the difficulty of isolating a purely astrocytic response to a physiological dopamine stimulus in organised brain tissue and recording any subsequent,

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potentially subtle, effects on neurons. Here perhaps lies one of the principal empirical obstacles in dissecting the physiological significance of dopamine-dependent signalling in astroglia.

Methodological issues

Much of the existing literature describing the mode of astrocytic response to dopamine is at odds, but there are some key experimental factors that may account for the variability of the findings in the literature.

Firstly, with the exception of cytoplasmic free Ca^{2+}, it is still unsure what the major output activity is in astroglia and hence what pathways to examine, and their relative importance to overall astrocyte physiology in the brain. When considering Ca^{2+} activity, astrocytes do not seem to show stereotyped responses to identical stimuli (this is certainly true for dopaminergic stimuli). In addition to precluding the signal averaging and hence recording noise reduction, this feature makes it difficult to know what parts of the Ca^{2+} signal are carrying information (if any), whether the signal is transmitted through the cell and what downstream signalling pathways it activates.

Secondly, astrocytes are extremely reactive cells and change both morphologically and biochemically when stressed or placed in a new environment within hours (Shao and McCarthy, 1993, Kimelberg et al., 1997). Therefore studying them in culture or in situ may fundamentally change the nature of their recorded response (most of the studies reported in this review will keep cells in culture for up to a week before experimenting). It is interesting to examine Ca^{2+} recordings from astrocyte primary cultures in comparison to astrocyte explant cultures (acutely isolated from brain tissue), in response to dopamine (Vaarmann et al., 2010). There is a clear difference, not only in basal Ca^{2+}+ activity, but also in the nature of the highly variable response to dopamine application. When considering morphology, many physiologically relevant responses to dopamine may take place in the highly ramified astrocytic end-feet that form in organised tissue in situ; studying astrocytes in culture, where their shape is drastically simplified, risks missing many possible forms of dopamine-induced astrocyte activity.Thirdly, there are no standardized protocols for stimulating astrocytes with dopamine. Dopamine and DAR agonist application concentration ranges from 1-100μM, and stimulation can be acute (phasic) or chronic (up to 24h). Also, given that the various imaging techniques, mRNA expression measurements and excretion measurement s have different sensitivities, no two studies are easily comparable. It is also a major concern when inferring how astrocytes might interact with dopamine in more physiological situations – dopamine input to different brain regions can be acute or tonic and generally reaches far lower concentrations than those used in the above studies (Iversen LL, 2010), how much can they therefore tell us about what is happening the living brain?

Finally, astrocytes are ubiquitous throughout the different brain regions but it is still uncertain to what extent their respective populations differ. In contrast, somata

of dopaminergic neurons are confined mainly to a small area of the midbrain – the Substantia Nigra and Ventral Tegmental Area – and project to spatially restricted areas of the basal ganglia, the frontal cortices and the limbic system (Iversen LL, 2010). As dopamine plays different roles in these functionally distinct areas, it is possible that any interaction uncovered between dopamine and astrocytes may be region-specific and thus not applicable across the whole astrocyte population. In this review we have been careful to state where different populations of astrocytes originate from – but given their extreme malleability in culture, it is unsure how much of their region-specific features they retain. Any reported regional heterogeneity in astrocyte-dopamine interaction (as in (Zanassi et al., 1999)), if robust, would be exceptionally interesting given that astrocytes are currently considered to form a relatively homogenous population.

Concluding remarks

We are only just beginning to uncover the interplay between the brain's astrocyte population and one of its most powerful neuromodulators, dopamine. It is clear that astrocytes can express dopamine receptors and transporters and that dopamine application can trigger profound intracellular changes in astrocytes. However, for the most part, evidence as to the mechanism of dopamine's actions is conflicting – due in part to inconsistent methodologies across the field, but also due to the inherent difficulty of studying both astrocytes and dopamine in the context of their action on neural networks. The study of astrocytic response to dopamine in situ has already begun and should go a long way to clarifying glial role in the dopaminergic modulation of neurotransmission. Once the major signalling pathways have been identified and clarified, the potential implications both therapeutic and for basic understanding of neuromodulation in the brain are enormous.

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