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Research article

Rat cortico-striatal sagittal organotypic slice cultures as *ex vivo* excitotoxic striatal lesion models

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ABSTRACT

Organotypic brain slice cultures are a useful tool to study neurological disease as they provide a 3-dimensional system which more closely recapitulates the *in vivo* cytoarchitectural complexity than standard 2-dimensional *in vitro* cell cultures. Building on our previously developed rat brain slice culture protocol, we have extended our findings to develop *ex vivo* excitotoxic lesion models by treatment of rat sagittal organotypic slices with AMPA or quinolinic acid (QA). We show that treatment of rat sagittal cortico-striatal organotypic slices with 8μM AMPA or 50μM QA causes striatal cell loss with a reduction in neuronal nuclei (NeuN)+ cells and an increase in ethidium homodimer-1 (EthD-1)+ dead cells compared to untreated slices. More specifically, following treatment with QA, we observed a reduction in medium spiny neuron DARPP32 + cells in the striatum and cortex of slices. Treatment of the slices with AMPA does not alter glial fibrillary acidic protein (GFAP) expression, while we observed an acute increase in GFAP expression 1-week post-QA exposure both in the cortex and striatum of slices. This recapitulates the excitotoxic and striatal degeneration observed in rat AMPA and QA lesion models *in vivo*. Our slice culture platform provides an advance over other systems with the ability to generate acute AMPA- and QA-induced striatal excitotoxicity in sagittal cortico-striatal slices which can be cultured long-term for at least 4 weeks. Our *ex vivo* organotypic slice culture system provides a long-term cellular platform to model neuronal excitotoxicity, with QA specifically modelling Huntington's disease. This will allow for mechanistic studies of excitotoxicity and neuroprotection, as well as the development and testing of novel therapeutic strategies with reduced cost and ease of manipulation prior to *in vivo* experimentation.

1. Introduction

The current knowledge regarding the pathogenesis of neurological disease has been gained predominantly from post-mortem human brain tissue and animal models of disease. To better our understanding of disease mechanisms and to identify and test potential therapeutic strategies, a range of disease models from *in vitro* cell cultures through to *in vivo* animal models have been developed which each have their benefits and limitations. Excitotoxicity, a term defined 40 years ago, is the neurotoxic effect that results from prolonged or excessive activation of excitatory amino acid receptors (Schwob et al., 1980). Excitotoxicity has been extensively studied and is implicated in many neurological diseases and conditions including Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, stroke, epilepsy, traumatic brain injury and spinal cord injury (Mehta et al., 2013). Glutamate is the most abundant excitatory neurotransmitter in the brain, mediating fast synaptic transmission. Glutamate binds to either ionotropic or metabotropic receptors. 2-amino-3-hydroxy-5-methyl-4-isoxazole propanoic acid (AMPA) is an ionotropic glutamate receptor which is often co-expressed synaptically with N-methyl-D-aspartate (NMDA) receptors and together these are involved in synaptic plasticity which underlies learning, memory and neuroprotection or alternatively, the induction of excitotoxicity. AMPA is an agonist at the AMPA receptor and mimics the excitatory effects of glutamate. Exposure to high concentrations of AMPA has been widely used in experimental models of neurotoxicity (Koh et al., 1990; May and Robison, 1993; Netzahualcoyotzi and Tapia, 2015).

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease affecting 1 in 15,000 individuals worldwide. HD is caused by a trinucleotide (CAG) repeat expansion mutation in exon one of the *Huntingtin* (*HTT*) gene which results in an elongated polyglutamine (PolyQ) tract at the N terminus on the HTT protein (O'Donovan, 1993).

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This genetic defect leads to a severe and gradual degeneration in the neostriatum and cerebral cortex, with a significant and preferential loss of GABAergic medium spiny neurons (MSN), the projection neurons of the striatum (Vonsattel et al., 1985). Clinically this degeneration underlies progressive motor dysfunction with typical choreatic movements, cognitive decline, psychiatric disturbances and progressive dementia (Martin and Gusella, 1986). Animal models of HD are used to test potential therapeutic approaches and amongst these is the well-characterised quinolinic acid (QA) lesion model. QA is a tryptophan metabolite and agonist at the NMDA receptor which at elevated concentrations is neurotoxic. Unilateral injection of QA into the striatum of adult rats results in the selective degeneration of striatal medium spiny neurons replicating the neurochemical characteristics underlying HD pathology (Beal et al., 1986).

In vitro cultures commonly involve 2-dimensional (2D) cell culture substrates which do not recapitulate the 3-dimensional (3D) complexity of the *in vivo* setting and have been shown to produce artificial cell morphologies and behaviours (Baker and Chen, 2012). *Ex vivo* organotypic slice cultures therefore bridge the gap between *in vitro* and *in vivo* systems allowing for easy and cost-effective manipulations in a conserved 3D environment that more closely mimics the *in vivo* cytoarchitecture, structural and synaptic features and extracellular cues. Hippocampal slices are most commonly utilised, but cortico-striatal organotypic slice cultures have been developed to model excitotoxicity *ex vivo*. In 1999, Kristensen et al. (1999) established a rat cortico-striatal excitotoxic *ex vivo* system that employs two different neurotoxic agents AMPA and kainic acid (KA). Cortico-striatal organotypic slices were prepared from 1-day-old rat pups and cultured at the air-membrane interface for 3–4 weeks before exposure to 1–6μM AMPA or 6–48 μM kA for 48 h (Kristensen et al., 1999). The neurotoxic effects of the various treatments was assessed by propidium iodide (PI) uptake, a measure of cell death, and revealed that both AMPA and KA are effective excitotoxic inducers. The degree of neuronal degeneration quantified by PI uptake and loss of GABA expression in cells directly correlated to the increasing dose of the agonists (Kristensen et al., 1999).

Other compounds have been used as excitotoxic agents to the more traditional AMPA, KA or NMDA, namely 2-amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl)propionic acid (ATPA), which at low concentrations is a selective KA receptor agonist, but at higher concentrations is an AMPA receptor agonist (Kristensen et al., 2001). Hippocampal slices from 5-7-day-old rats were used to assess the excitotoxic effects of ATPA in comparison to AMPA, KA and NMDA. These slices were shown to be a useful platform to better understand the mechanisms underlying excitotoxicity. What is more, hippocampal slices displayed excitotoxic patterns more closely mimicking that seen *in vivo* than primary neuronal cultures, highlighting the advantage of slice cultures as a more relevant model (Kristensen et al., 2001).

Very few studies have reported the generation of an *ex vivo* QA-induced striatal lesion in cortico-striatal organotypic slice cultures. In 1991, Schurr and colleagues showed that exposure of acute rat hippocampal slices to 100μM–500μM QA for 30 min resulted in neuronal damage but that the lower concentrations required a combination of hypoxia for measurable neuronal loss to be achieved (David et al., 1996). The same year, Matyja and colleagues cultured rat hippocampal organotypic slices for 21 days and then exposed the tissue to 100μM QA with or without the calcium channel block, nimodipine, which was found to be partially protective of QA-induced damage up to 7 days post-exposure (Matyja and Kida, 1991). A similar study investigating the neuroprotective activity of kynurenine 3-mono-oxygenase inhibitors on 1–10μM QA-treated and oxygen and glucose deprived organotypic hippocampal slices, found that the QA treatment prevented the neuroprotective activity (Carpenedo et al., 2002). Based on these studies, neurotoxicity has been modelled in acute slices by incubation of striatal slices in 100μM of QA for a minimum of 3 h (Tasset et al., 2010). One study reported the generation of a chronic excitotoxic *ex vivo* model by exposure of rat cortico-striatal organotypic slices to 100nM QA for up to 7 weeks (Whetsell Jr and Schwarcz, 1989). The 1,000-fold lower concentration used resulted in a slowly progressive chronic model of neuronal degeneration providing a valuable mechanism by which to better study and understand QA and its mechanisms (Whetsell Jr and Schwarcz, 1989).

Taken together these studies have predominantly utilised rat hippocampal slices but have also developed rat cortico-striatal organotypic slices which, following treatment with either AMPA or QA, can provide *ex vivo* platforms of excitotoxicity. These later systems use coronal slices of the frontal cortex and striatum and predominantly demonstrate only short-term culture survival, limiting their application in testing novel neuroprotective agents or cell replacement strategies. Nevertheless, these systems can be used to investigate the mechanisms underlying neurotoxicity induced by the various compounds and to study the short-term neuroprotective effects of various agents.

The current study builds on our established chronic rat sagittal organotypic slice culture system (McCaughey-Chapman and Connor, 2017). We have shown that rat sagittal organotypic slice cultures can remain viable with a conserved 3D cytoarchitecture for up to 6 weeks in culture. Treatment of slices with 20mM 6-hydroxydopamine with 1μM desipramine generated *ex vivo* DA cell loss system which mimics the progressive pathology of Parkinson's disease (McCaughey-Chapman and Connor, 2017). In the current study, we have extended our findings using: 1) AMPA to induce an excitotoxic lesion; and 2) QA to produce a striatal lesion relevant to the neuronal cell loss seen in HD. We show that rat sagittal cortico-striatal organotypic slices can be used as a long-term *ex vivo* model of excitotoxicity and striatal cell loss relevant in HD.

2. Materials and methods

2.1. Animals

Postnatal 8- to 10-day old male and female Sprague-Dawley rat pups were used in this study. All animals were housed in a 12-hour light-dark cycle with access to food and water ad libitum. Animal euthanasia was designed in accordance with the New Zealand Animal Welfare Act 1999 and had approval from the University of Auckland Animal Ethics Committee. All efforts were made to minimise the number of animals used.

2.2. Sagittal brain organotypic slice culture generation and culturing

Sagittal brain organotypic slice cultures were generated and cultured as previously described (McCaughey-Chapman and Connor, 2017). Briefly, animals were euthanised by decapitation and the brains divided into two hemispheres for sectioning. 300μm-thick sagittal brain slices were cut using a vibratome [Leica Biosystems] in ice-cold medium consisting of Advanced DMEM/F-12 with 1% penicillin-streptomycin [Thermo Fisher Scientific, #12634010 and #15140148]. Individual slices were mounted onto sterile membrane inserts in 6-well plates [Corning, #COR3450] and cultured at the air-membrane interface at 35 °C with 5% CO_2. Slices were cultured in MEM with Hanks balanced salts [Thermo Fisher Scientific, #11575032], 1% penicillin-streptomycin and 25% horse serum [Thermo Fisher Scientific, #16050130] for 3 days (1mL of medium added below the membrane insert). To limit glial scar formation, a cocktail of three mitotic inhibitors was added to the medium for the first three days of culturing: uridine, 5-fluorodeoxyuridine and cytosine-ß-arabinofuranoside [4.4mM each, Sigma, #U3003, #F0503 and #C1768]. The slices were then transitioned into a serum-free medium consisting of Advanced DMEM/F-12 with 2% B-27 supplement [Thermo Fisher Scientific, #17504044] and 1% N-2 supplement [Thermo Fisher Scientific, #17502048] and cultured for up to 4 weeks (Timeline summarised in Figure 1).

2.3. AMPA and QA treatment

At Day 3 of culturing, slices were divided into three groups: untreated, AMPA-treated or QA-treated (Figure 1). To induce an excitotoxic

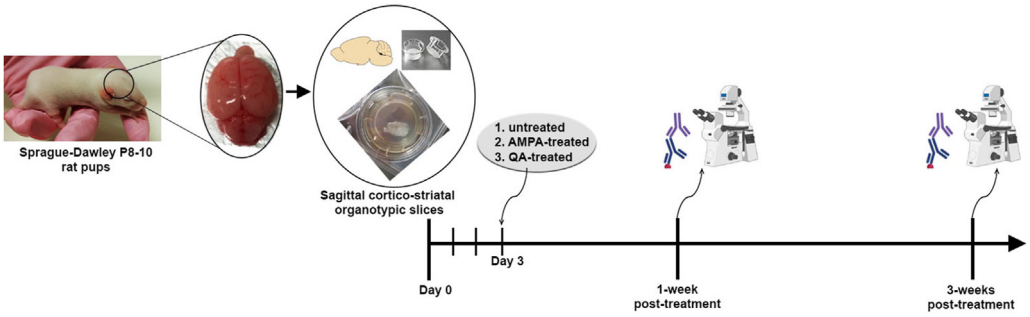

Figure 1. Schematic diagram depicting the methodology and timeline of the study.

lesion, the slices were incubated for 24 h in 8μM AMPA [Sigma, #05164-50MG] based on the study by Bernardino and colleagues (Bernardino et al., 2005). To mimic the loss of medium spiny striatal neurons that occurs in Huntington's disease, a separate set of slices was treated for 24 h with 50μM QA [Sigma, #P63204]. This was based on previous studies that have induced an acute lesion by incubation of slices in 100μM QA for 3–12 h (Sofroniew, 2009) and correlates with the concentration of QA we have used *in vivo* (Kells et al., 2004). Thereafter slices were cultured for an additional 1- or 3-weeks (Figure 1).

2.4. Ethidium homodimer-1 live tissue staining

Cell viability was assessed in the striatum of slices 1- and 3-weeks post-treatment (either untreated, AMPA-treated or QA-treated, n = 6 slices per time point and per treatment) by live tissue staining with the dead cell marker, ethidium homodimer-1 (EthD-1) [Thermo Fisher Scientific, #E1169]. As previously described, the slices were incubated in 4μM EthD-1 for 40 min at room temperature, at the desired time point, then rinsed with PBS and immediately imaged to avoid fluorescence signal degradation (McCaughey-Chapman and Connor, 2017).

2.5. Immunohistochemistry

Slices were fixed in 4% paraformaldehyde for 24 h at 4 °C. Whole mount slices were stained using a shortened version of the optical clearing technique iDISCO (Renier et al., 2014). Briefly, after 24 h of permeabilisation, the slices were blocked and then incubated in primary antibody for 48 h. The extent of neuronal cell loss, medium spiny neuron loss and astrocyte number was assessed by quantification of the expression of NeuN [1:250, MAB377], DARPP32 [1:200, Creative Diagnostics] and GFAP [1:500, Dako Z0334], respectively (n = 6 slices per time point and treatment for each stain). Alexa Fluor conjugated secondary antibodies were used to fluorescently label the antigens of interest and following dehydration in a series of increasing concentrations of methanol and clearing in DBE, the membrane-bound slices were placed onto a glass microscope slide and imaged using a Nikon inverted fluorescent microscope and Zeiss LSM 710 inverted confocal microscope.

2.6. Quantification and statistical analysis

Images were captured throughout the striatum for all stains, and the cortex for DARPP32 and GFAP staining. The number of EthD-1+, NeuN+ and DARPP32 + cells was quantified using a custom particle analyser macro in ImageJ which involved consistent thresholding of the images, removing the same level of background and incorporating the watershed function to segment close proximity particles into individual particles. The particle analysis was then defined by size and pixel circularity. The extent of GFAP staining was assessed by measuring the integrated density in ImageJ. The number of EthD-1+, NeuN+ and DARPP32 + cells and GFAP + density was calculated for the striatum or cortex in each slice. An average per time point and treatment group was calculated (n = 6 slices from n = 6 rats). The data was reported as a percentage of the expression in 1-week treated slices (EthD-1) or as a percentage of 1-week untreated slices (NeuN, DARPP32 and GFAP). Statistical significance was determined using raw values and a two-way ANOVA. As treatment and time each only contain two groups, post-hoc analysis was only performed in the case of a significant interaction, in which case independent sample T-tests were conducted.

3. Results

3.1. AMPA induces neuronal excitotoxicity

Treatment of the slices with AMPA did not lead to any visible shrinkage, swelling or other changes in the macroscopic appearance of the tissue. Cell viability was assessed in the striatum of AMPA-treated organotypic slices at 1 week and 3 weeks post-treatment and in corresponding control slices using the dye and dead cell marker, ethidium homodimer-1 (EthD-1). EthD-1 staining in the striatum of cortico-striatal organotypic slices appeared more abundant in the AMPA-treated slices, at both time points, with only a few EthD-1+ cells visible in the untreated slices (Figures 2B and 2C). Quantification of striatal EthD-1 staining demonstrated that AMPA treatment induced a significant increase in striatal EthD-1 staining both at 1 week and 3 weeks post-exposure, when compared to corresponding untreated slices, with a mean EthD-1 expression in untreated slices at 1 week of 41.09% ± 7.75% of the average EthD-1 expression in AMPA-treated slices at 1 week and a mean EthD-1 expression in untreated slices at 3 weeks of 67.63% ± 6.52% of the average EthD-1 expression in AMPA-treated slices at 1 week (Figure 2D). There was no difference in EthD-1 staining in AMPA-treated slices at 1 week or 3 weeks post-treatment (100% ± 16.6% and 123.86 ± 29.51%, respectively), as determined by a significant effect of treatment on mean EthD-1 expression ($F(1,20) = 10.614$, $p = 0.004$), with no significant interaction ($F(1,20) = 0.006$, $p = 0.94$), nor simple main effect of time ($F(1,20) = 2.034$, $p = 0.169$). This shows that a 24-hour exposure to 8μM AMPA is sufficient to induce an excitotoxic lesion in rat organotypic slices.

Immunohistochemical characterisation of the cell loss in response to AMPA treatment revealed significant neuronal loss with no change in astrocyte expression. Indeed, abundant neuronal nuclei (NeuN) were seen in the untreated slices, with a few present in the AMPA-treated slices (Figures 3B and 3C). Quantification of striatal NeuN expression showed that AMPA treatment induced a significant decrease in striatal NeuN + cells both at 1 week and 3 weeks post-exposure, when compared to the corresponding untreated slices, with a mean NeuN expression in AMPA-treated slices at 1 week of 38.09% ± 8.2% of the average NeuN expression in untreated slices at 1 week and a mean NeuN expression in AMPA-treated slices at 3 weeks of 36.77% ± 5.51% of the average NeuN expression in untreated slices at 1 week (Figure 3D). There was no difference in the mean striatal NeuN expression in untreated slices over time (100% ± 19.52% and 109.03% ± 9.92%, respectively), as demonstrated by a significant effect of treatment on mean NeuN expression ($F(1,20) = 31.197$, $p = 0.0001$), with no significant interaction ($F(1,20) = 0.186$, p

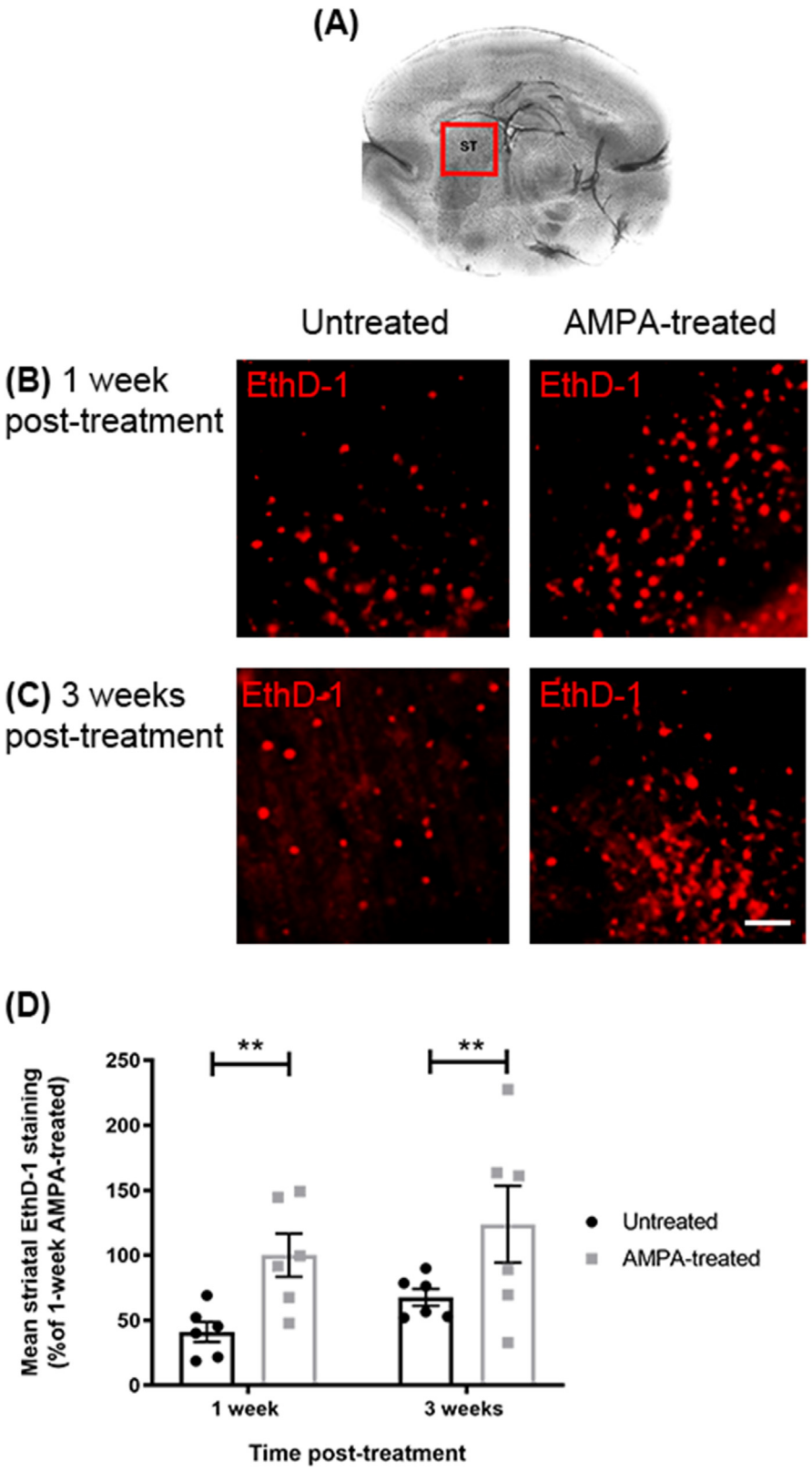

Figure 2. Treatment of slices with 8μM AMPA induces a significant increase in striatal EthD-1 staining. (A) Schematic outlining the area of analysis. (B–C) Few EthD-1+ cells are visible in untreated slices at both 1- and 3-weeks post-treatment, while abundant EthD-1 expression can be seen in AMPA-treated slices at both time points. Scale: 100μm. (D) Quantification of the mean EthD-1 staining in untreated and AMPA-treated slices at 1- and 3-weeks post-treatment. Data are presented as a percentage of EthD-1 staining in AMPA-treated slices after 1 week. Data represent mean ± SEM with n = 6. Statistical significance was determined by two-way ANOVA with $p = 0.004$ for the effect of treatment on mean EthD-1 staining.

= 0.671), nor simple main effect of time ($F(1,20) = 0.103$, $p = 0.751$). When considering the glial fibrillary acidic protein (GFAP)+ expression in the slices (Figure 4A – C), there was no difference in the mean GFAP + expression in response to AMPA treatment, or in regard to time (1 week untreated: 100% ± 35.1%; 1-week AMPA-treated: 95.2% ± 16.59%; 3 weeks untreated: 145.51% ± 37.91%; 3 week AMPA-treated: 105.78% ± 51.11%; treatment: $F(1,19) = 0.349$, $p = 0.562$; time: $F(1,19) = 0.553$, $p = 0.466$; interaction: $F(1,19) = 0.215$, $p = 0.648$) (Figure 4D). These findings reinforce the observations made with the EthD-1 expression, whereby exposure of cortico-striatal slices to 8μM AMPA for 24 h induced a sustained excitotoxic neuronal lesion in the slices with no effect on astrocytes.

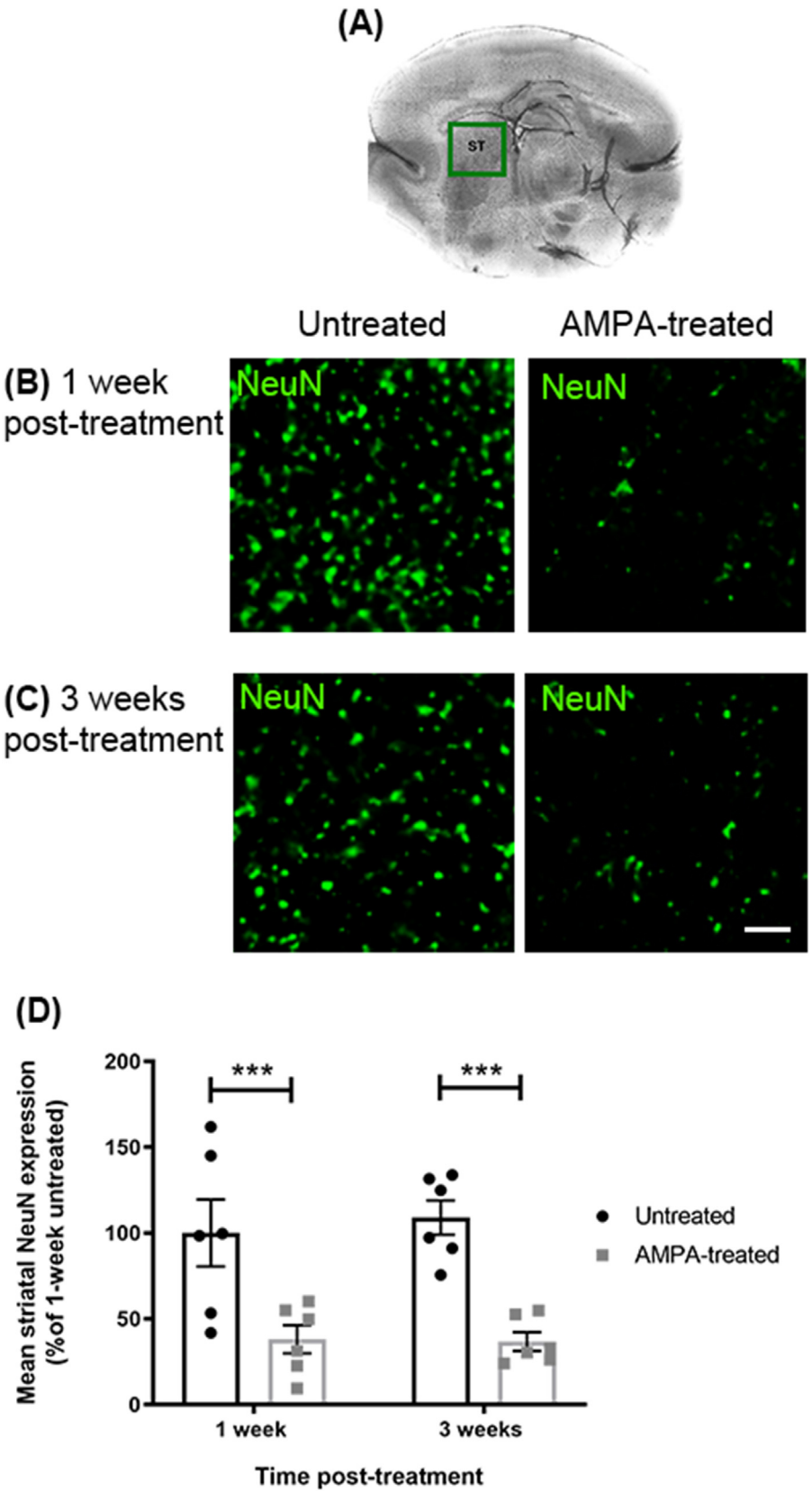

Figure 3. Treatment of slices with 8μM AMPA induces a significant reduction in striatal NeuN expression. (A) Schematic outlining the area of analysis. (B–C) Abundant NeuN expression is visible in untreated slices at both time points, while few NeuN + nuclei are seen in AMPA-treated slices. Scale: 100μm. (D) Quantification of the mean NeuN expression in untreated and AMPA-treated slices at 1- and 3-weeks post-treatment. Data are presented as a percentage of NeuN expression in untreated slices after 1 week. Data represent mean ± SEM with n = 6. Statistical significance was determined by two-way ANOVA with p = 0.0001 for the effect of treatment on mean NeuN expression.

3.2. Quinolinic acid induces medium spiny neuron loss and an acute reactive astrocyte response

No visible shrinkage, swelling or other changes in the macroscopic appearance of the tissue were noted in response to QA treatment. To investigate the effect of treating cortico-striatal slices with 50μM QA, EthD-1 staining was assessed at 1- and 3-weeks post-exposure in untreated and QA-treated slices. Some EthD-1+ cells were visible in untreated slices with more abundant EthD-1 staining in QA-treated slices (Figures 5B and 5C). Quantification of striatal EthD-1 staining revealed a

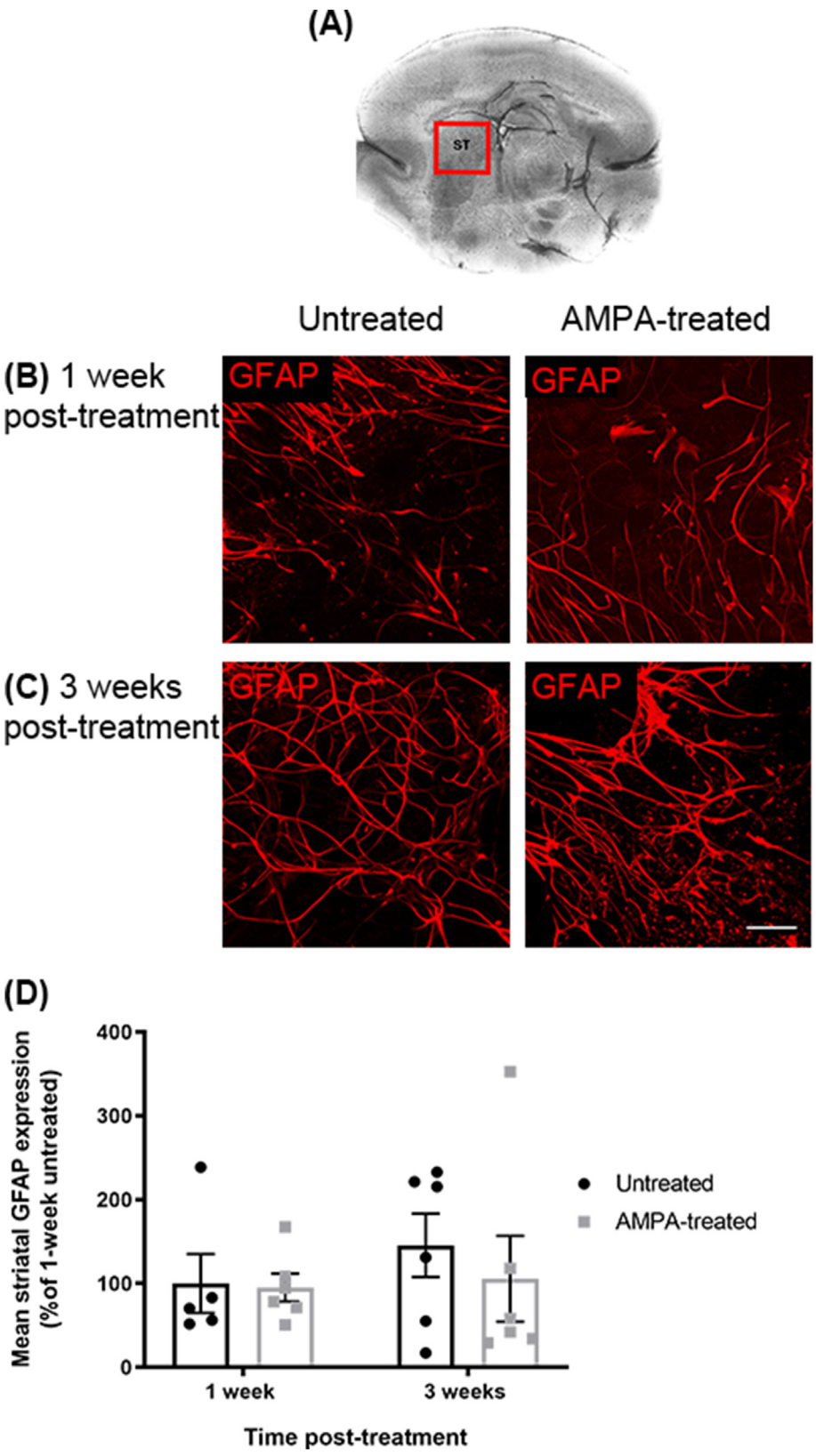

Figure 4. Treatment of slices with 8μM AMPA does not alter GFAP expression. (A) Schematic outlining the area of analysis. (B–C) Abundant GFAP expression is visible in untreated and AMPA-treated slices at both time points, Scale: 150μm. (D) Quantification of the mean GFAP expression in untreated and AMPA-treated slices at 1- and 3-weeks post-treatment. Data are presented as a percentage of GFAP expression in untreated slices after 1 week. Data represent mean ± SEM with n = 6. No statistical significance was determined by two-way ANOVA.

significant increase in EthD-1 expression following treatment with QA, irrespective of time, with a mean EthD-1 expression in untreated slices at 1 week of 41.68% ± 13.31% of the average EthD-1 expression in QA-treated slices at 1 week and the mean EthD-1 expression in untreated slices at 3 weeks of 44.38% ± 14.47% of the average EthD-1 expression in QA-treated slices at 1 week (Figure 5D). There was no difference in the mean EthD-1 staining in response to QA exposure at 1 week or 3 weeks post-treatment (100% ± 18.83% and 143.28% ± 34.61%, respectively), as demonstrated by a significant effect of treatment on mean EthD-1 stain ($F(1,20) = 12.744$, $p = 0.002$), with no significant interaction ($F(1,20)$

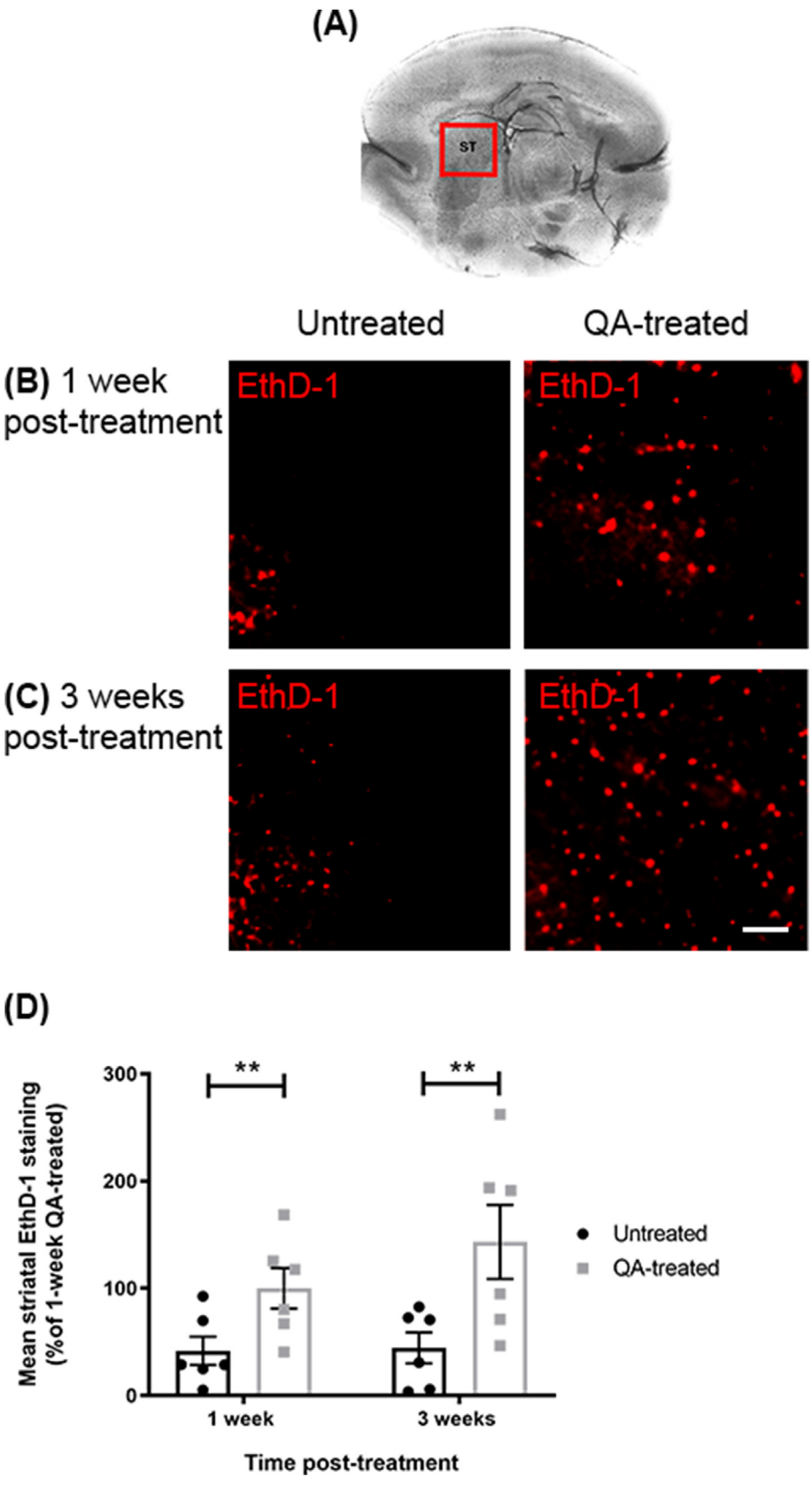

Figure 5. Treatment of slices with 50μM QA induces a significant increase in striatal EthD-1 staining. (A) Schematic outlining the area of analysis. (B–C) Few EthD-1+ cells are visible in untreated slices at both 1- and 3-weeks post-treatment, while abundant EthD-1 staining can be seen in QA-treated slices at both time points. Scale: 100μm. (D) Quantification of the mean EthD-1 staining in untreated and QA-treated slices at 1- and 3-weeks post-treatment. Data are presented as a percentage of EthD-1 staining in QA-treated slices after 1 week. Data represent mean ± SEM with n = 6. Statistical significance was determined by two-way ANOVA with p = 0.002 for the effect of treatment on mean EthD-1 staining.

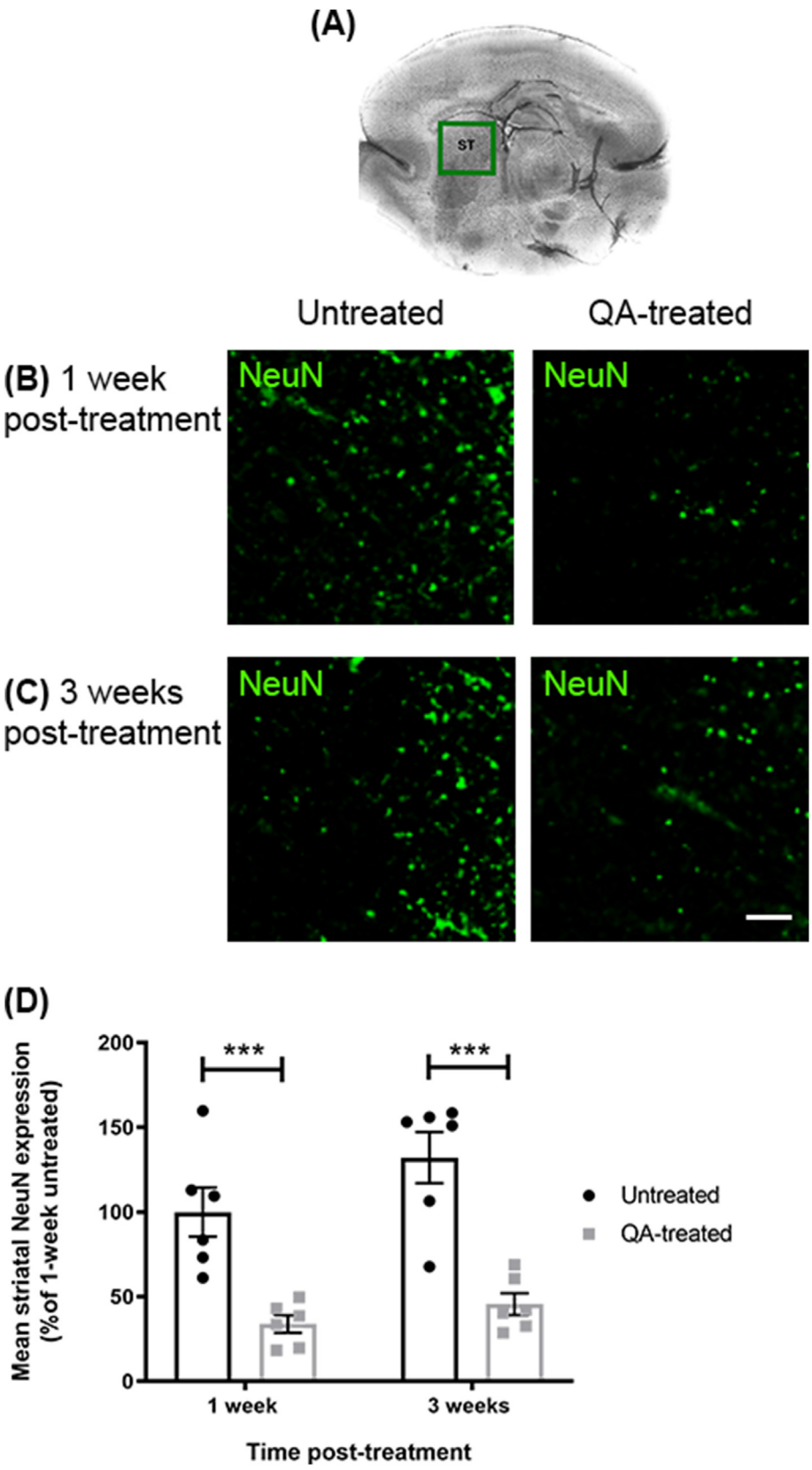

Figure 6. Treatment of slices with 50μM QA induces a significant reduction in striatal NeuN expression. (A) Schematic outlining the area of analysis. (B–C) Abundant NeuN expression is visible in untreated slices at both 1- and 3-weeks post-treatment, while few NeuN + cells can be seen in QA-treated slices at both time points. Scale: 100μm (D) Quantification of the mean NeuN expression in untreated and QA-treated slices at 1- and 3-weeks post-treatment. Data are presented as a percentage of NeuN expression in untreated slices after 1 week. Data represent mean ± SEM with n = 6. Statistical significance was determined by two-way ANOVA with p = 0.0001 for the effect of treatment on mean NeuN expression.

= 0.849, p = 0.368), nor simple main effect of time (F (1,20) = 1.090, p = 0.309). These results indicate that a 24-hour exposure to 50μM QA induces a striatal lesion in cortico-striatal organotypic slices.

Immunohistochemical characterisation of the neuronal and astrocytic cell populations in response to QA treatment revealed a significant and sustained reduction in NeuN + neurons and specifically of DARPP32 + medium spiny neurons. At the same time, QA exposure induced an acute increase in GFAP + astrocytes. Indeed, abundant NeuN expression was present in the striatum of untreated slices, with few NeuN + nuclei visible in QA-treated slices (Figures 6B and 6C). Quantification of striatal NeuN

expression showed that QA treatment induced a significant decrease in NeuN + nuclei both at 1 week and 3 weeks post-exposure, when compared to the corresponding untreated slices, with a mean NeuN expression in QA-treated slices at 1 week of 33.83% ± 5.16% of the average NeuN expression in untreated slices at 1 week and a mean NeuN expression in QA-treated slices at 3 weeks of 45.61% ± 6.48% of the average NeuN expression in untreated slices 1 week (Figure 6D). There was no difference in the average NeuN expression in untreated slices at 1 week (100% ± 14.52%) and 3 weeks (132.13% ± 15.13%), as shown by a significant effect of treatment on mean NeuN expression ($F(1,20) = 45.832$, $p = 0.0001$), with no significant interaction ($F(1,20) = 0.815$, $p = 0.378$), nor simple main effect of time ($F(1,20) = 3.790$, $p = 0.066$).

As administration of QA *in vivo* results in medium spiny neuron neurotoxicity, the effect of QA treatment on DARPP32 expression was investigated both in the striatum and cortex of slices. Fewer DARPP32 + cells were visible in both the striatum and cortex of QA-treated slices (Figure 7A – C and 7E - G). Quantification of the number of DARPP32 + cells demonstrated a reduction in the mean striatal DARPP32 expression in QA-treated slices at both 1 week (27.24% ± 17.42%) and 3 weeks (22.9% ± 7.44%) post-treatment when compared to untreated slices at both time points (1 week: 100% ± 30.83%; 3 weeks 62.28% ± 21.44%) (Figure 7D). This sustained loss of DARPP32 + cells was demonstrated by a significant effect of treatment ($F(1,18) = 6.439$, $p = 0.021$), with no significant interaction ($F(1,18) = 0.571$, $p = 0.460$), nor simple main effect of time ($F(1,18) = 0.906$, $p = 0.354$). The same was found in the cortex, with the mean cortical DARPP32 expression significantly reduced at both 1 week (44.03% ± 6.07%) and 3 weeks (43.04% ± 14.08%) post-QA when compared to untreated slices (1 week: 100% ± 28.89%; 3 weeks: 100.44% ± 15.03%) (Figure 7H). Again, a statistically significant effect of treatment supported these findings ($F(1,18) = 8.638$, $p = 0.009$), with no significant interaction ($F(1,18) = 0.001$, $p = 0.971$), nor simple main effect of time ($F(1,18) = 0.001$, $p = 0.989$). These findings are in accordance with the EthD-1 expression results, demonstrating that treatment of rat cortico-striatal organotypic slices with 50μM QA for 24 h induces a sustained loss of medium spiny neurons.

The effect of QA exposure on GFAP + astrocytes was investigated and abundant GFAP + staining was present throughout the striatum and cortex of slices 1 week after QA treatment (Figure 8A - B and E - F). Quantification of the GFAP + immunostaining density revealed a significant interaction between treatment and time (Striatum: $F(1,18) = 7.304$, $p = 0.015$; Cortex: $F(1,17) = 5.478$, $p = 0.032$). Subsequent post-hoc analysis demonstrated an increase in mean GFAP expression in QA-treated slices, 1-week post-exposure, in both the striatum (317.46% ± 38.43%, $t(8) = -4.178$, $p = 0.003$) and cortex (310% ± 46.32%, $t(7) = -2.490$, $p = 0.042$) (Figures 8D and 8H). This increase in striatal and cortical GFAP expression returned to levels comparable to untreated

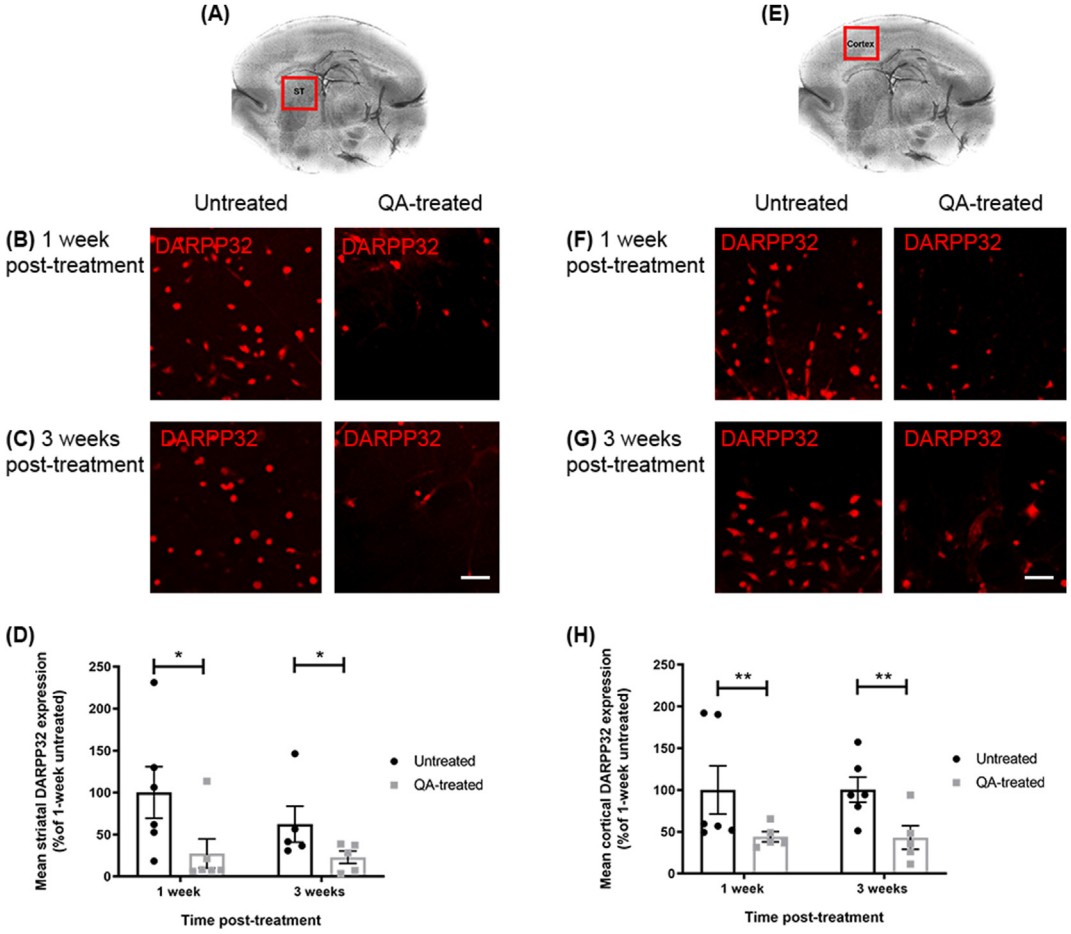

Figure 7. Treatment of slices with 50μM QA induces a significant reduction in striatal and cortical DARPP32 expression. (A and E) Schematic outlining the area of analysis. (B–C and F-G) Abundant DARPP32 expression is visible in untreated slices at both 1- and 3-weeks post-treatment, while few DARPP32 + cells can be seen in QA-treated slices at both time points. Scale: 100μm. (D) Quantification of the mean striatal DARPP32 expression in untreated and QA-treated slices at 1- and 3-weeks post-treatment. Data are presented as a percentage of striatal DARPP32 expression in untreated slices after 1 week. Data represent mean ± SEM with n = 6. Statistical significance was determined by two-way ANOVA with $p = 0.021$ for the effect of treatment on mean striatal DARPP32 expression. (H) Quantification of the mean cortical DARPP32 expression in untreated and QA-treated slices at 1- and 3-weeks post-treatment. Data are presented as a percentage of cortical DARPP32 expression in untreated slices after 1 week. Data represent mean ± SEM with n = 6. Statistical significance was determined by two-way ANOVA with $p = 0.009$ for the effect of treatment on mean cortical DARPP32 expression.

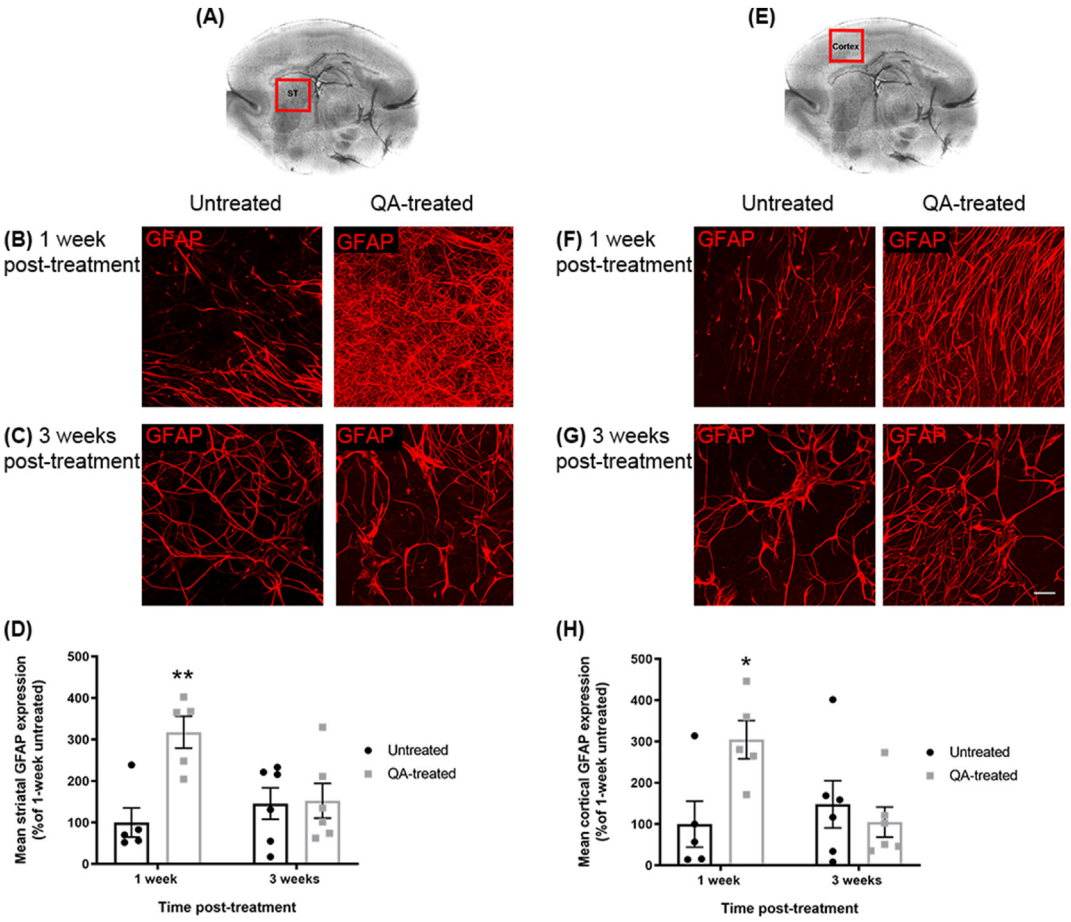

Figure 8. Treatment of slices with 50μM QA induces an acute increase in striatal and cortical GFAP expression. (A and E) Schematic outlining the area of analysis. (B–C and F-G) GFAP + cells are visible in untreated slices at both time points and in QA-treated slices after 3 week, while an abundant GFAP expression can be seen in QA-treated slices after 1 week. Scale: 300μm. (D) Quantification of the mean striatal GFAP expression in untreated and QA-treated slices at 1- and 3-weeks post-treatment. Data are presented as a percentage of striatal GFAP expression in untreated slices after 1 week. Data represent mean ± SEM with n = 6. Statistical significance was determined by two-way ANOVA with a significant interaction of time and treatment ($p = 0.015$) and subsequent independent sample T-test. (H) Quantification of the mean cortical GFAP expression in untreated and QA-treated slices at 1- and 3-weeks post-treatment. Data are presented as a percentage of cortical GFAP expression in untreated slices after 1 week. Data represent mean ± SEM with n = 6. Statistical significance was determined by two-way ANOVA with a significant interaction of time and treatment ($p = 0.032$) and subsequent independent sample T-test.

slices by 3 weeks post-treatment (striatum: 152.2% ± 41.63%; cortex: 104% ± 36.41%).

4. Discussion

Organotypic cortico-striatal slice cultures have been developed to study cellular mechanisms and provide a more complex and relevant 3D platform than 2D cell cultures for neurotoxicity studies, disease modelling and drug testing. Although *ex vivo* slices do not completely recapitulate the *in vivo* setting as sectioning of the tissue results in disrupted cellular connections, processes and interactions, they are nevertheless an advance on *in vitro* cultures as they incorporate a more complex cytoarchitecture closely mimicking the *in vivo* environment. In this study, we built on our previous findings by establishing rat sagittal cortico-striatal organotypic slice cultures that can be treated with either AMPA or the NMDA receptor agonist QA to generate striatal excitotoxic lesions *ex vivo*. We demonstrated that a 24-hour exposure to 8μM AMPA induced a significant and sustained increase in the number of EthD-1+ cells and a decrease in the number of NeuN + nuclei in the striatum of organotypic cortico-striatal slices from 1-week post-treatment. Treatment of cortico-striatal slices with 8μM AMPA did not alter GFAP expression in the slices. Bernardino and colleagues treated hippocampal slices with 8μM AMPA for 24 h and cell death was measured by propidium iodide (PI) uptake every 24 h for 3 days only. They observed basal PI uptake levels in control untreated slices were below 50% that of AMPA-treated slices, which is comparable to our findings with EthD-1 (Bernardino et al., 2005). In another study, rat cortico-striatal slice cultures were exposed to 1–6μM AMPA for 48 h and consequential cell death measured by PI uptake (Kristensen et al., 1999). They found that 3μM and 6μM AMPA induced a considerable increase in PI uptake, which was not observed when a 1μM dose of AMPA was used. Basal levels of PI uptake were very low at about 10–20%. They also showed that a 6μM dose of AMPA induced ~50% neuronal cell loss in the cortico-striatal slices 48 h post-treatment (Kristensen et al., 1999). This indicates that our findings are comparable to those made in other *ex vivo* studies, however our system provides an advance as we maintained our AMPA-treated cortico-striatal organotypic slices up to 3 weeks post-lesion, thereby providing an acute excitotoxic rat cortico-striatal *ex vivo* platform for longitudinal studies. Little is known about the effect of AMPA on GFAP + reactive astrocytes. In this study, we observed no change in GFAP expression over time following a 24-hour exposure of 8μM AMPA. It has been shown that treatment of mouse primary GFAP + astrocytes with up to 500μM AMPA had no effect on the astrocyte cultures, whereas exposure to 500μM kainate resulted in a gradual degeneration of the astrocytes (David et al., 1996). It follows that the lack of astrocyte toxicity in response to AMPA exposure could be due to the reduced glial expression of AMPA receptors and perhaps that AMPA receptors in astrocytes undergo desensitisation upon stimulation (David et al., 1996). No study has looked at the effect of

AMPA treatment on astrocyte expression in organotypic slices, which renders our system a more comprehensive excitotoxic system.

As previously mentioned, there are no reports of QA-induced acute excitotoxic cortico-striatal organotypic slice culture systems, which makes our findings an advance in the field providing a unique *ex vivo* striatal neurotoxic lesion model. Chronic exposure of rat cortico-striatal organotypic slices to 100nM QA for up to 7 weeks resulted in a focal degeneration characterised by vacuoles, swollen dendrites and post-synaptic densities and neuronal cell death (Whetsell Jr and Schwarcz, 1989). *In vivo*, Beal and colleagues demonstrated that a unilateral injection of QA resulted in marked depletion of GABA and substance P in the striatum 2 weeks post-injection (Beal et al., 1986). This loss was shown to be dose-dependent with increasing concentrations of QA, 75–450nM, resulting in more severe depletion of substance P, ~55%–~10% and GABA, ~75%–25% (Beal et al., 1986). In agreement, we have shown that injection of 50nM QA into the adult rat striatum results in a 42% loss of NeuN + neurons *in vivo* (Kells et al., 2004). This highlights that the extent of neuronal loss in the cortico-striatal organotypic slice cultures following 24 h treatment with 50μM QA (~34% and ~46% at 1-week and 3-weeks post-QA respectively) is comparable to that observed in the QA-lesioned animal model of Huntington's disease.

Interestingly, we observed an acute reactive astrocyte response to QA treatment in our slices which was widespread through the striatum and cortex at 1-week post-QA only. In response to an insult, astrocytes can undergo reactive astrogliosis which is characterised by a molecular and morphological change (Sofroniew, 2009). The degree of reactive astrogliosis is dependent on the nature and severity of the insult. Astrocytes express NMDA receptors which have been shown to be functional and involved in neuronal-glial signal transmission (Schipke et al., 2001; Lalo et al., 2006). QA, an NMDA receptor agonist, has also been shown to enhance synaptosomal glutamate release and decrease glutamate uptake into astrocytes, resulting in an increased extracellular glutamate concentration which can lead to overstimulation of the glutamatergic system and hence promotion of neurotoxicity (Tavares et al., 2002). In addition, QA can induce brain inflammation through the stimulation of chemokine and chemokine receptor expression in astrocytes (Guillemin et al., 2003). *In vivo*, intracranial injection of 50 g/L QA results in a striatal astrogliosis characterised by increased GFAP density at 7 days post-QA injection which was sustained in the striatum for up to a year post-injection (Björklund et al., 1986). We propose that the 50μM concentration of QA used in our study induces a less severe excitotoxic lesion in the slices which is characterised by an acute reactive astrogliosis response at 1-week post-QA exposure which diminishes over time.

Overall, both the AMPA- and QA-lesioned cortico-striatal *ex vivo* systems will provide excellent novel platforms for future longitudinal studies investigating the mechanisms underlying excitotoxicity in neurological conditions, for the development and testing of potential neuroprotective agents and for the testing of cell replacement strategies prior to advancing to more time-consuming, expensive and complex *in vivo* animal studies.

Declarations

Author contribution statement

Amy McCaughey-Chapman: Conceived and designed the experiment; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bronwen Connor: Conceived and designed the experiment; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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Some of the diagrams were sourced from BioRender.com.

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