

**COMMITTEE FOR AID AND EDUCATION IN NEUROCHEMISTRY (CAEN) CATEGORY 1B:  
REPORT for Research supplies for use in the applicant's home laboratory -  
International Society for Neurochemistry  
ISN CAEN Grants - April 2016 round**

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Huntington's Disease (**HD**) is a neurodegenerative genetic disorder with a prevalence of 5-10/100.000 worldwide and the rate of occurrence is highest in descendants from western European people. HD is caused by a CAG repeat expansion in the huntingtin (**Htt**) gene that codes for polyglutamine in Htt protein. Mutant Htt accumulates over time and is toxic to neurons, particularly to medium spiny neurons in the striatum [1]. At present, management of HD is limited to treatment of the hyperkinetic movement disorder and psychiatric problems but no treatment modifies the course of the disease. Thus, treatments that slow or prevent disease progression in HD are a major need. Astrocytes are glial cells that exert many essential and complex physiological functions and can become reactive in response to diverse pathological conditions in the brain. Mutant Htt expressed only in mouse astrocytes induces HD-like neurologic symptoms [4]. This suggests that astrocyte may contribute to HD pathogenesis and that they can become new targets for disease treatment. Recently, it was reported that conditioned medium (**CM**) from astrocytes in culture protected striatum neurons in an animal model of HD [5]. Microglia are the tissue-resident macrophages of the brain and they serve immune surveillance, apoptotic clearance, neurogenesis, and synaptic pruning functions [6]. Microglial cells can also be activated by injury and secrete harmful and/or beneficial molecules that influence neuron survival. In a mouse model of HD, microglia become activated and produce higher levels of pro-inflammatory cytokines than macrophages from the same animals [7]. Thus, the inflammatory response may be involved in HD pathogenesis. Indeed, pro-inflammatory cytokines are elevated in the striatum of HD patients [8]. However, astrocytic or microglial contribution to this response in HD is not well characterized. Reactive astrocytes and microglia are considered detrimental for neurons and this can be true in chronic neuroinflammation. But it is also recognized that glial cells are fundamental in tissue repairing processes and neuron survival. Expression of Htt is ubiquitous and is higher in the brain than in other tissues. Glial cells can also be affected by mutant Htt expression and can contribute to disease progression. Thus, increasing glial viability in neurodegeneration may be a new neuroprotective approach to treat HD. Mitochondrial dysfunction is observed in HD patients and systemic administration of 3-nitropropionic acid (**3-NP**), an inhibitor of succinate dehydrogenase from mitochondrial complex II, induces HD-like symptoms in animals and humans [9]. Brain-derived neurotrophic factor (**BDNF**) is a neurotrophin that promotes neuron development and survival. BDNF has an anti-apoptotic action in neurons and protective effects on several neurodegenerative diseases like HD [10]. Striatal neurons are dependent on BDNF for their survival and reduced striatal BDNF expression has been observed in HD patients and in HD animal models [11]. Therefore, reduced BDNF expression is proposed to underlie selective striatal cell death in HD. The effects of BDNF have been well studied on neurons but very little is known about its role on astrocytes and microglia.

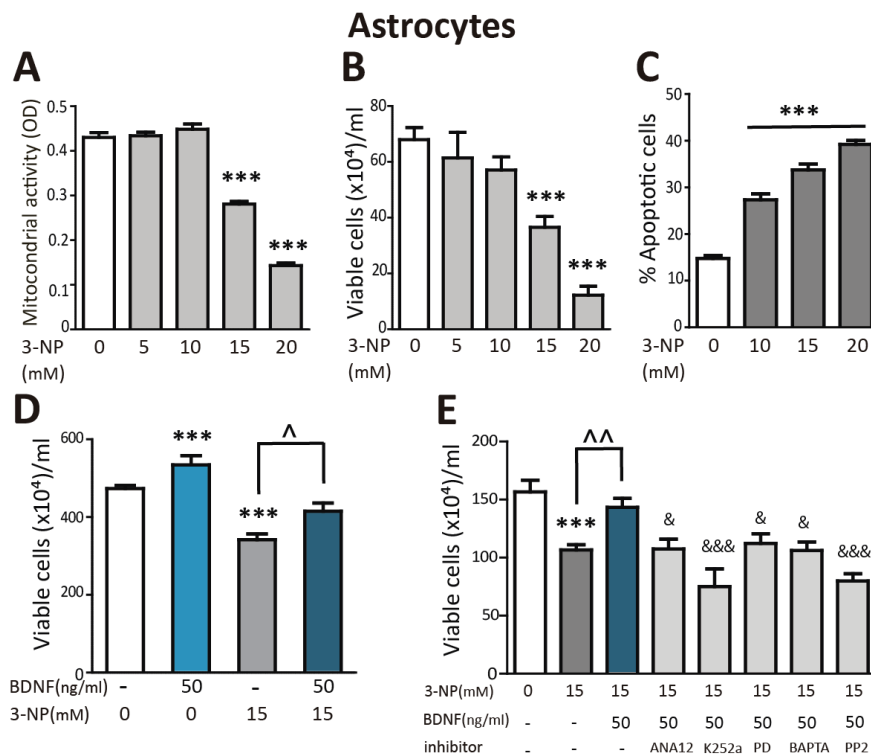
#### **HYPOTHESIS**

Astrocytes and microglia perform several functions that are fundamental for brain physiology. Nevertheless, their participation in neurodegenerative diseases has recently begun to be explored. Since astrocyte and/or microglia can promote neuron survival, they could become targets for new treatments to delay or stop neurodegeneration. Our preliminary data shows that BDNF reduces astrocyte cell death induced by 3-NP. The effect of BDNF on microglia viability is unknown. Therefore, we will study BDNF effect on astrocyte and microglia viability in an *in vitro* model of HD. Moreover, our preliminary data also show that CM from BDNF-treated astrocytes fully prevents neuronal death induced by 3-NP. Thus, our project will look into the protective mechanisms exerted by BDNF through astrocytes and also possibly by microglia in a model of HD.

## SPECIFIC AIMS

### 1- To investigate the *in vitro* effect of BDNF on viability and inflammatory response of astrocyte and microglia treated with 3-NP. To evaluate neuron, astrocyte and microglia interactions.

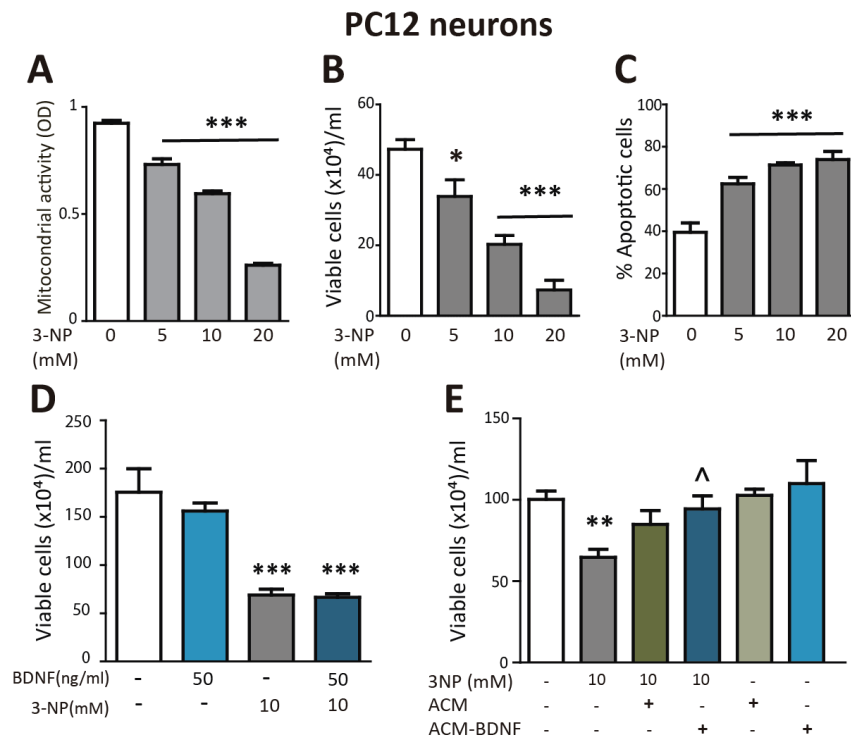
Our previous results showed that BDNF increased astrocyte viability and prevented death by serum deprivation (Saba et al. 2017, manuscript in revision). This protective effect is exerted through TrkB-T1 which is abundantly expressed in astrocytes. TrkB-T1 activation in astrocytes induces ERK and Src phosphorylation, an effect blocked by the calcium chelator BAPTA (Saba et al. 2017, manuscript in revision). 3-NP inhibited mitochondrial activity (Fig. 1A), decreased viability (Fig. 1B) and increased apoptosis (Fig. 1C) of astrocytes. BDNF prevented death of astrocytes induced by 3-NP (Fig. 1D). The protective effect of BDNF was blocked by inhibition of TrkB (ANA-12, K252a) ERKs (PD98059), calcium (BAPTA) and Src (PP2) (Fig. 1E).



**Figure 1. BDNF protects astrocytes from 3-NP-induced cell death.**

\*\*\* $p < 0.001$  vs. control;  $\wedge p < 0.05$  and  $\wedge\wedge p < 0.01$  vs. 3-NP; &  $p < 0.05$  y &&&  $p < 0.001$  vs. 3-NP+BDNF.

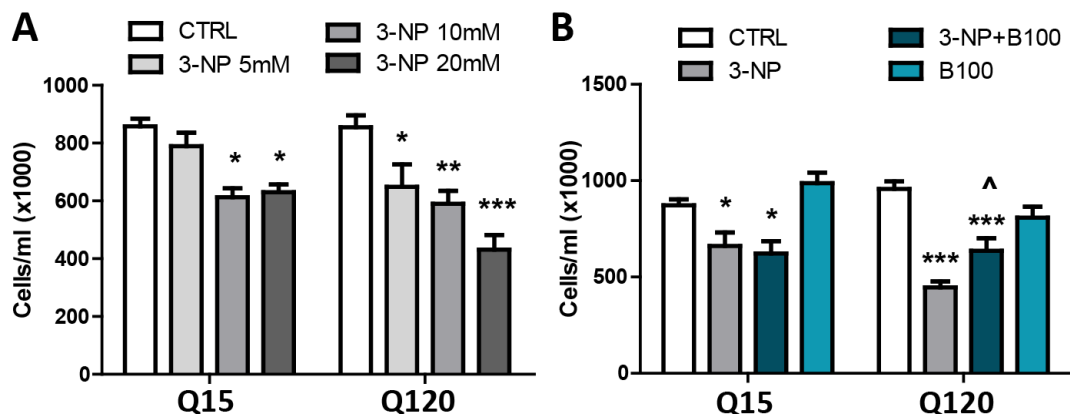
3-NP also inhibited mitochondrial activity (Fig. 2A), decreased viability (Fig. 2B) and increased apoptosis (Fig. 2C) of PC12 neurons (Fig. 2A-C). Concordantly with the fact that PC12 cells don't express BDNF receptor TrkB, BDNF treatment did not exert a protective effect in these cells directly (Fig. 2D). However, CM from control astrocytes (ACM) partially protected, whereas ACM from BDNF-treated astrocytes (ACM-BDNF) completely abolished the reduction in viability induced by 3-NP (Fig. 2E).



**Figure 2. ACM from BDNF-treated astrocytes blocked PC12 death by 3-NP.**

\*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 vs control. ^p<0.05 vs. 3-NP.

We next examined whether BDNF exerts protective effects in a cellular model of HD. **ST14A** cells derive from embryonic rat striatal cells that express normal human Htt with 15 CAG repeats (**Q15**) or mutant human Htt with 120 CAG repeats (**Q120**). We found that Q120 cells were more susceptible to death induced by 3-NP than Q15 cells since 20 mM 3-NP kills 50% of Q120 cells but 20% of Q15 (Fig. 3A). We evaluated BDNF effects in these cells. BDNF protected Q120 cells from death induced by 3-NP (Fig. 3B) whereas it had no effect on Q15 cells viability. Together data indicates that BDNF selectively protects Q120 cells from 3-NP-induced death.

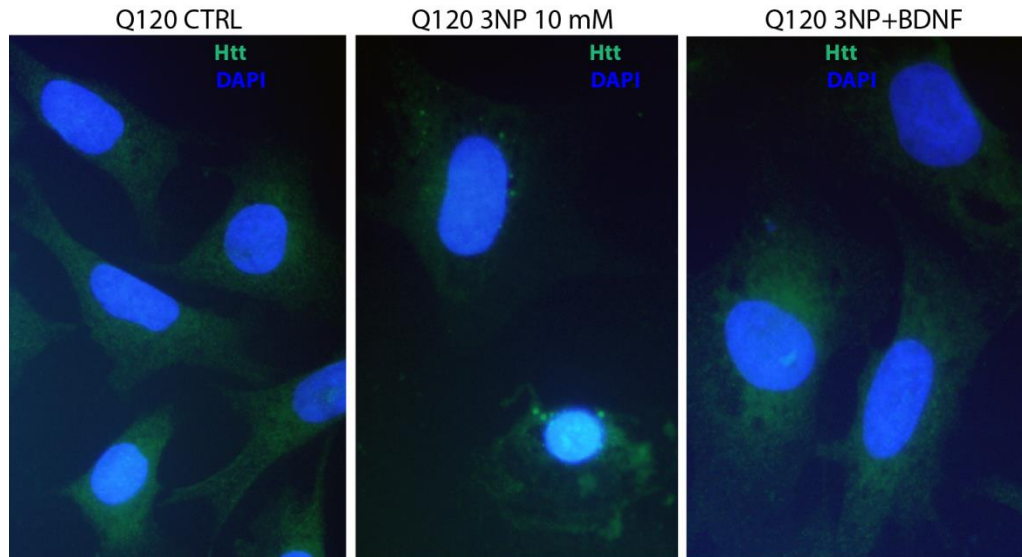


**Figure 3. BDNF protects ST14A-Q120 neurons from death by 3-NP.**

\*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 vs control. ^p<0.05 vs. 3-NP.

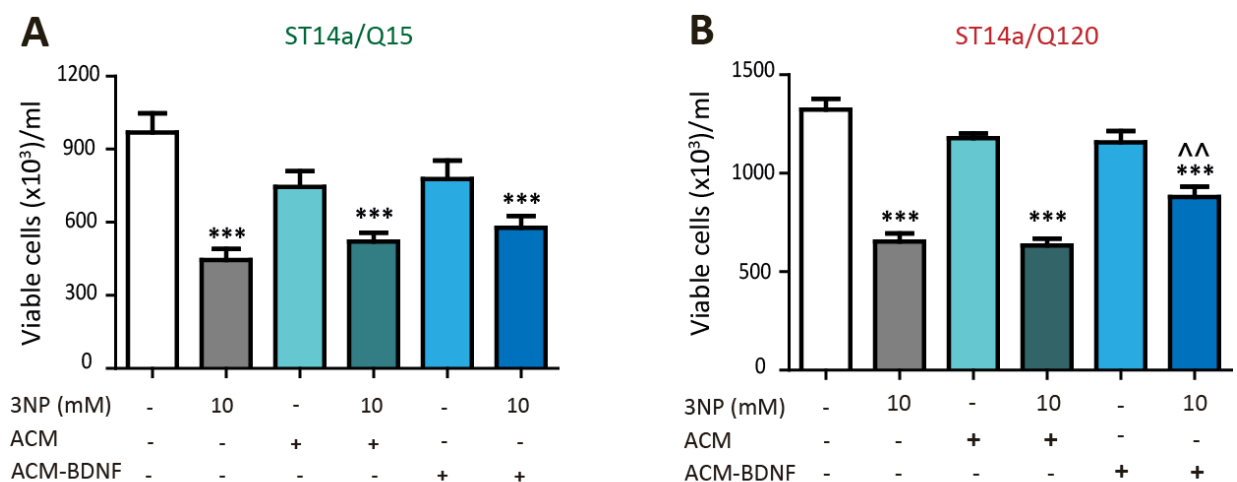
Since Htt can form perinuclear aggregates in cells that may alter nuclear envelope causing death of striatal neurons [24], and cytoplasmic aggregates can correlate with neurodegeneration in HD [25], we next

evaluated Htt expression in ST14A Q15 and Q120 by immunocytochemistry. We found cytoplasmic expression of Htt in both Q15 and Q120 control cells. However, 3-NP induced cytoplasmic aggregates of Httm in Q120 cells but not in Q15 cells. Notably, BDNF prevented the formation of Htt aggregates induced by 3-NP (Fig. 4), an effect that correlates with BDNF being selectively protective in Q120 cells (Fig. 3B).



**Figure 4. BDNF prevents Httm aggregates induced by 3-NP in Q120 cells.**

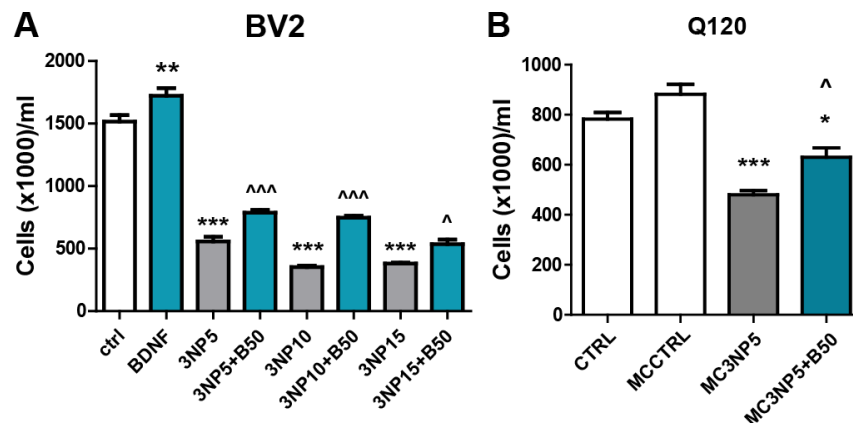
In order to investigate the interaction between central nervous system cells, we determined the effect of conditioned medium (CM) of astrocytes or BV2 microglial cells treated with BDNF on neuron survival. First, we determined the effect of CM from astrocytes on Q15 and Q120 viability. We found that ACM from control or from BDNF-treated astrocytes (ACM-BDNF) did not modify the effect of 3-NP on Q15 viability (Fig. 5A). Conversely, while ACM from control astrocytes did not exert protection on 3-NP-induced cell death of Q120 cells, ACM-BDNF protected Q120 cells from 3-NP-induced cell death (Fig. 5B).



**Figure 5. ACM from BDNF-treated astrocytes protects ST14A-Q120 from death by 3-NP.**

\*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 vs control. ^^p<0.01 vs. 3-NP.

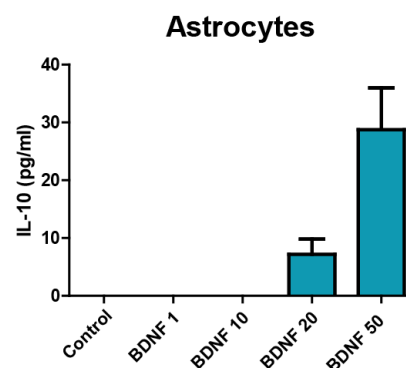
On the other hand, we investigated BDNF effects on BV2 microglial cells treated with 3-NP. BV2 cell viability was diminished by 3-NP (Fig. 6A). BDNF by itself increases BV2 viability and also protects BV2 cells from 3-NP-induced cell death (Fig. 6A). CM from BV2 cells treated with 3-NP for 24 h reduced Q120 cell viability whereas CM from BV2 control cells did not modify Q120 cell viability (Fig. 6B). Moreover, BV2 cells treated for 24 h with 3-NP plus BDNF reduced the inhibitory effect of CM-3NP on Q120 viability (Fig. 6B), suggesting that BDNF treatment of microglia can ameliorate their toxic effect.



**Figure 6. BDNF protects BV2 cells from 3-NP induced cell death leading to neuroprotection.**

A) \*\*p<0.01 \*\*\*p<0.001 vs. Ctrl and ^p<0.05, ^^p<0.001 vs 3-NP. B) \*p<0.05, \*\*\*p<0.001 vs. Ctrl and ^p<0.05 vs MC3-NP.

We are starting to investigate BDNF effects on the inflammatory response of astrocytes and microglia. Preliminary data indicates that BDNF increases astrocyte IL-10 levels in a dose dependent fashion (Fig. 7).



**Figure 7. BDNF induces an increase in IL-10 levels in astrocytes.**

## CONCLUSIONS

HD is a fatal disease and understanding HD pathogenesis and the action of protective agents is of the outmost importance. BDNF expression is known to be decreased in neurodegenerative disorders such as HD. Thus, increasing BDNF levels is a very attractive approach that has proved to be effective in *in vitro* and *in vivo* models. However, little is known about BDNF effects on astrocytes and microglia. Our present data shows for the first time that BDNF has protective effects on astrocytes and microglia. Furthermore, we demonstrated that glial cells treated with BDNF secrete protective factors that prevent neuron death in HD. These data help reveal BDNF mechanism of action in neuroprotection in the hope of developing new strategies to deal with neurodegenerative diseases.

## PURCHASES

We spent the funds of the CAEN grant in buying ST14A-Q15 and Q120 cells, a model of HD that was not available in Argentina. We also purchased anti-Htt antibody and cell culture reagents.

## SCIENTIFIC PUBLICATIONS DERIVED FROM THE GRANT.

### 1) ISN-ESN Meeting, 20-24 AUGUST 2017, Paris, France.

J. Saba, D. Ramírez, J. Turati, L. Carniglia, D. Durand, M. Lasaga, **C. Caruso**. “*TrkB receptor mediates BDNF protection of astrocytes*”.

### 2) 2ND FALAN CONGRESS 17-20 OCTOBER 2016, Buenos Aires, Argentina.

J. Saba, D. Ramírez, J. Turati, L. Carniglia, D. Durand, M. Lasaga, **C. Caruso**. “*Brain-derived neurotrophic factor exerts antioxidant and protective effects on astrocytes and neurons treated with 3-nitropropionic acid*”.

**3) J. Saba, D. Ramírez, J. Turati, L. Carniglia, D. Durand, M. Lasaga, C. Caruso. 2017, “Astrocyte truncated TrkB mediates BDNF antiapoptotic effect leading to neuroprotection”, manuscript in revision.**

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