Chondroitinase and antidepressants promote plasticity by releasing TRKB from dephosphorylating control of PTPσ in parvalbumin neurons

Angelina Lesnikova[1], Plinio Cabrera Casarotto[1], Senem Merve Fred[1], Mikko Voipio[1], Frederike Winkel[1,#a], Anna Steinzeig[1], Hanna Antila[1,#b], Juzoh Umemori[1], Caroline Biojone[1]*, Eero Castrén[1]*.

Affiliations:

[1]Neuroscience Center, HiLIFE, University of Helsinki, Helsinki, Finland

[#a]Current Address: Department of Developmental Neurobiology, King's College London, London, UK

[#b]Current Address: Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, PA, USA

*co-corresponding authors:

Caroline Biojone: caroline.biojone@helsinki.fi

Eero Castrén: eero.castren@helsinki.fi

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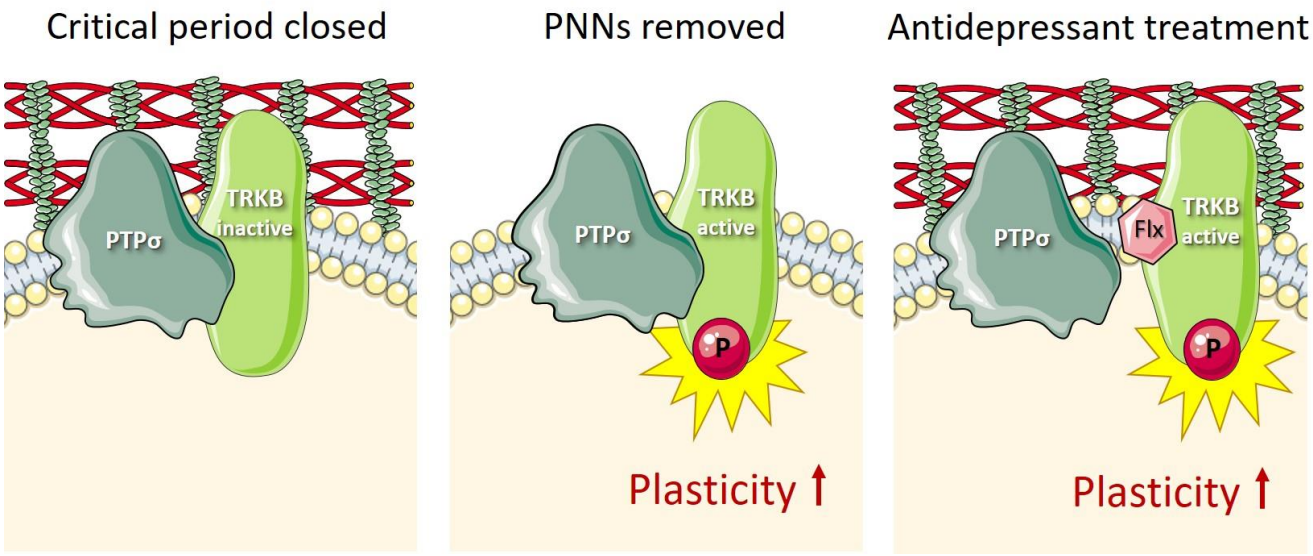
Abstract

Perineuronal nets (PNNs) are an extracellular matrix structure rich in chondroitin sulphate proteoglycans (CSPGs) which preferentially encase parvalbumin-containing (PV^+) interneurons. PNNs restrict cortical network plasticity but the molecular mechanisms involved are unclear. We found that reactivation of ocular dominance plasticity in the adult visual cortex induced by chondroitinase (chABC)-mediated PNN removal requires intact TRKB signaling in PV^+ neurons. Additionally, we demonstrate that chABC increases TRKB phosphorylation (pTRKB), while PNN component aggrecan attenuates BDNF-induced pTRKB in cortical neurons in culture. We further found that protein tyrosine phosphatase sigma (PTPσ, PTPRS), receptor for CSPGs, interacts with TRKB and restricts TRKB phosphorylation. PTPσ deletion increases phosphorylation of TRKB *in vivo* and *in vitro*, and juvenile-like plasticity is retained in the visual cortex of adult PTPσ deficient mice ($PTP\sigma^{+/-}$). The antidepressant drug fluoxetine, which is known to promote TRKB phosphorylation and reopen critical period-like plasticity in the adult brain, disrupts the interaction between TRKB and PTPσ by binding to the transmembrane domain of TRKB. We propose that both chABC and fluoxetine reopen critical period-like plasticity in the adult visual cortex by promoting TRKB signaling in PV^+ neurons through inhibition of TRKB dephosphorylation by the PTPσ-CSPG complex.

Graphical abstract



Chondroitinase and antidepressant treatment promotes plasticity by releasing TRKB from dephosphorylating control of PTPσ. In the presence of PNNs, PTPσ actively dephosphorylates TRKB and suppresses its signaling (left). In the absence of PNNs, PTPσ is inactive and TRKB phosphorylation is facilitated (middle). Fluoxetine disrupts TRKB:PTPσ interaction allowing TRKB signaling (right).

Introduction

Plasticity is the ability of the brain to change itself in terms of establishing new neuronal connections and rewiring existing ones. Plasticity is prominent in early life and it peaks during so-called "critical periods" when the ability of the brain to change and adapt is at its highest [1]. After the end of the critical period, plasticity persists but at significantly diminished levels [2].

Closure of the critical periods is thought to be mediated by changes in cortical excitatory/inhibitory (E/I) balance that take place due to maturation of cortical inhibitory interneurons. Fast-spiking interneurons expressing parvalbumin (PV^+) orchestrate synchronous neuronal oscillations and play a particularly important role in this process [3]. Closure of the critical period coincides with the functional maturation of PV-positive cells and establishment of perineuronal nets (PNNs) around them [4–6]. Perineuronal nets are mesh-like structures of the extracellular matrix that surround the somata and proximal dendrites of certain types of neurons, particularly PV^+ interneurons, in the central nervous system [7]. Chondroitin sulfate proteoglycans (CSPGs), such as aggrecan and brevican, are major components of PNNs.

After the closure of a critical period, neuronal plasticity can still be modulated, and critical period-like plasticity can be induced in the adult brain by a number of different methods [8,9]. Digestion of perineuronal nets by chABC treatment has been demonstrated to induce ocular dominance plasticity in the adult visual cortex [10]. Local chondroitinase injections into different brain areas have also been shown to promote recovery of spinal cord injury [11] and extinction of fear memories in adult rodents [12–16]. However, the mechanisms through which chABC influences plasticity in the central nervous system (CNS) remain unclear.

Another way to induce a critical period-like plasticity in the adult brain is antidepressant treatment. Antidepressants have been shown to activate neurotrophic receptor tyrosine kinase 2 (TRKB), the receptor for brain-derived neurotrophic factor (BDNF), and promote plasticity through its signaling pathways [6,17–20]. Fluoxetine, a widely prescribed antidepressant, induces ocular dominance plasticity in the rodent visual cortex [21] and makes fear-related memories in mice susceptible to erasure [22]. We have recently found that the activation of TRKB in PV^+ interneurons is both necessary and sufficient for antidepressant-induced plasticity in the mature CNS [23].

ChABC and antidepressant treatment exert similar plasticity-promoting effects in the adult brain; however, it is not known whether they recruit similar molecular mechanisms or share signaling pathways. We hypothesize that receptor-like protein tyrosine phosphatase sigma (PTPσ) might be a nexus that mediates plasticity processes induced by both methods. PTPσ is a receptor for chondroitin sulfate proteoglycans (CSPGs) [24], and it has been demonstrated to be essential for the inhibitory effects of CSPGs on neurite outgrowth [24–26]. It has also been shown that PTPσ interacts with and modulates the activity of TRK receptors [27], including TRKB [28], through dephosphorylation.

We now demonstrate that TRKB activity in PV^+ interneurons is essential for plasticity induced through PNN digestion by chABC. While CSPGs removal by chABC injection into the visual cortex promotes ocular dominance shift, this effect is abolished in heterozygous mice with reduced TRKB in parvalbumin-positive neurons (PV-$TRKB^{+/-}$). We have also confirmed that PTPσ interacts with TRKB, and genetic deficiency of PTPσ promotes TRKB phosphorylation (pTRKB) *in vitro* and *in vivo*. We further show that PTPσ deficiency promotes plasticity at network levels, as $PTP\sigma^{+/-}$ mice display critical period-like plasticity in the visual cortex in adulthood. Finally, we have observed that the antidepressant fluoxetine disrupts TRKB:PTPσ interaction *in vitro* and *in vivo*. Taken together, our data suggest that chABC and antidepressant treatment converge on the same pathway involving increased signaling of TRKB in PV^+ interneurons.

Results

TRKB signaling in PV$^+$ neurons is essential for chondroitinase ABC-induced plasticity

Digestion of PNNs by chABC is a well-known activator of plasticity [29], and it has been shown to induce critical period-like plasticity in the adult rodent visual cortex through temporary degradation of CSPGs in the extracellular matrix [10]. However, precise molecular mechanisms responsible for the plasticity-promoting effect of chABC remain largely unknown.

We tested the hypothesis that TRKB functioning in PV$^+$ interneurons may be involved in chABC-induced plastic changes. For that purpose, we generated mice heterozygous for full-length TRKB allele in PV$^+$ interneurons (PV-TRKB$^{+/-}$) [30]. We assessed the ability of chABC to promote ocular dominance plasticity in their visual cortex. Four-month old mice underwent assessment of their ocular dominance by optical imaging of the intrinsic signal (IOS1) [31,32]. After the first imaging session, the mice were injected with chABC or vehicle into the binocular area of the visual cortex followed by monocular deprivation for 7 days. As expected, vehicle-treated wild-type mice failed to show any OD plasticity in the second imaging session right after the monocular deprivation, whereas wild-type mice treated with chABC showed a clear shift in the ocular dominance towards the non-deprived eye (Fig. 1a), as previously shown [10]. However, PV-TRKB$^{+/-}$ mice failed to show any shift in ocular dominance regardless of whether they were treated with chABC or not (Fig. 1a). These data demonstrate that chABC-mediated reactivation of visual cortical plasticity is dependent on intact TRKB signaling in the PV$^+$ interneurons.

Next, we assessed whether PNN digestion with chABC induces TRKB activation in primary cortical neurons. Primary neurons cultured for 7 days *in vitro* already present detectable PNN deposition, as seen in sup. fig. 1. We treated rat cortical neurons with 2 U/ml chABC for 30 min and checked the activation of TRKB by assessing its phosphorylation levels (pTRKB) [33] (Fig 1b). We observed that reduction of PNNs by chABC treatment robustly increased pTRKB *in vitro*.

After observing that PNN digestion with chABC treatment positively affects pTRKB, we investigated whether treatment with aggrecan, a major PNN component in the adult CNS, might have an opposing effect on pTRKB and render BDNF-induced TRKB activation less effective. We added 10 µg/ml aggrecan to the primary cortical neurons cultured for 6 DIV. After 24 hours, we challenged the cells with 20 ng/ml BDNF for 10 min. Aggrecan treatment did not alter basal pTRKB levels (that are normally very low *in vitro*), however, it significantly decreased BDNF-induced pTRKB (Fig. 1c). Taken together, these data demonstrate that PNNs regulate plasticity in a TRKB-dependent manner *in vivo* and that PNNs exert negative effects on TRKB activation *in vitro*.

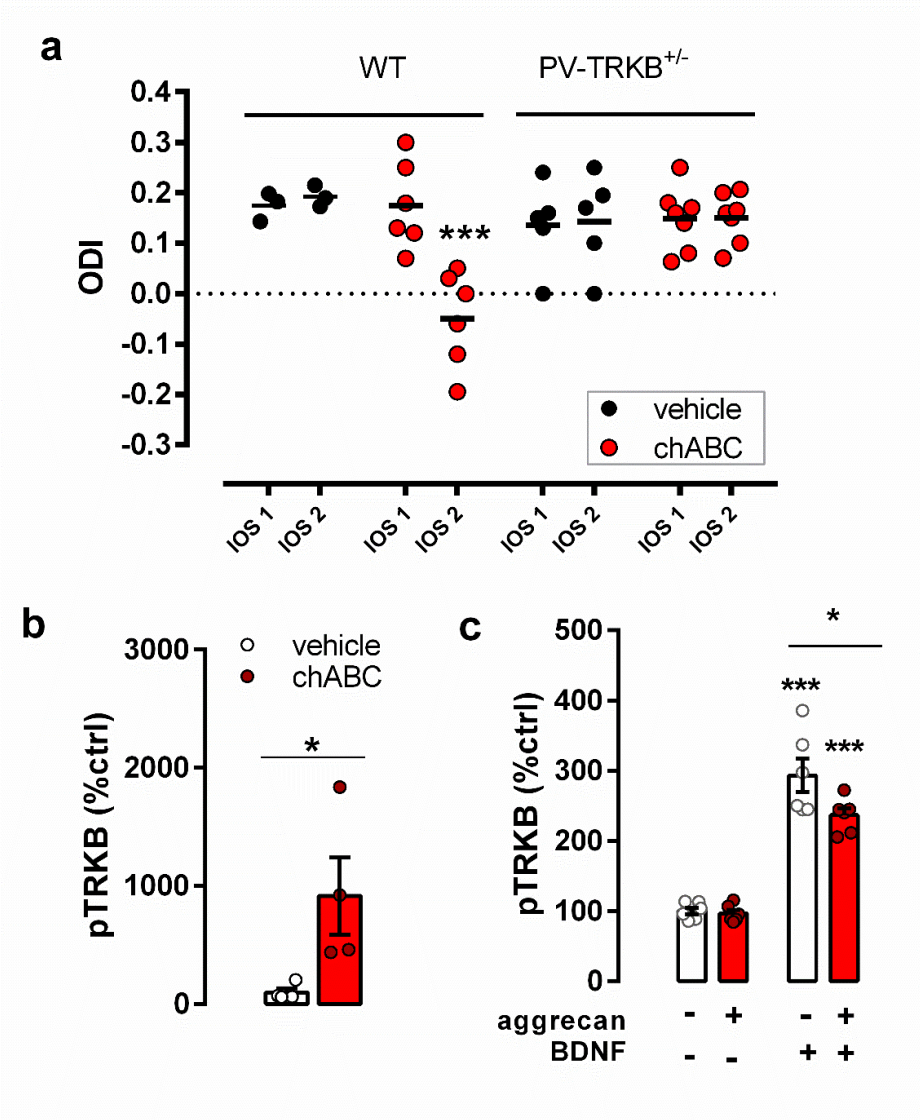


Fig. 1: ChABC-induced plasticity requires intact TRKB signaling in PV⁺ neurons. a) PNN removal by local chABC injection reopens critical period-like plasticity in the visual cortex of WT but not in PV-TRKB$^{+/-}$ mice. b) 30 min chABC treatment in cortical neurons (7 DIV) derepress TRKB phosphorylation. c) Supplementation of Neurobasal medium with CSPG aggrecan attenuates BDNF-induce TRKB phosphorylation in cortical neurons. Columns and bars represent mean±sem, respectively, and scattered points represent individual values. Data was analyzed by three-way ANOVA and Tukeys´s post-hoc (a), Mann-Whitney (b), or two-way ANOVA followed by Tukey´s multiple comparison test (c). *p<0.05, **p<0.005, ***p<0.0005.

Deletion of CSPG receptor PTPσ extends the critical period and facilitates TRKB activation

PTPσ is a recognized inhibitor of neuronal plasticity and a receptor for CSPGs, and previous data indicate that PTPσ interacts with TRKB [28]. We therefore hypothesized that PTPσ might restrict TRKB signaling through dephosphorylation in PNN-bearing PV⁺ interneurons and thereby inhibit plasticity. To confirm TRKB:PTPσ interaction, NIH3T3 cell line stably expressing either TRKA or TRKB (MG.TRKA and MG.TRKB, respectively), were transfected with myc-PTPσ, immunoprecipitated with anti-TRKB antibody and blotted for PTPσ. In the transfected samples, we observed a band below 250 kDa (Fig. 2a), which is consistent with the predicted molecular weight of the plasmid´s product. Additionally, a 165 kDa band was observed in non-transfected cells, which corresponds to the endogenous mature full-length PTPσ [27]. When the membrane was stripped and

reblotted for TRKB, we observed a band of 140 kDa corresponding to the full-length TRKB. This band was seen in TRKB expressing cells only, which rules out potential unspecific binding of TRKB antibodies to TRKA receptors. These data indicate that PTPσ interacts with TRKB. Furthermore, our previous proteomic study found PTPσ among the proteins that were immunoprecipitated with TRKB in hippocampal samples of the adult mouse brain [34].

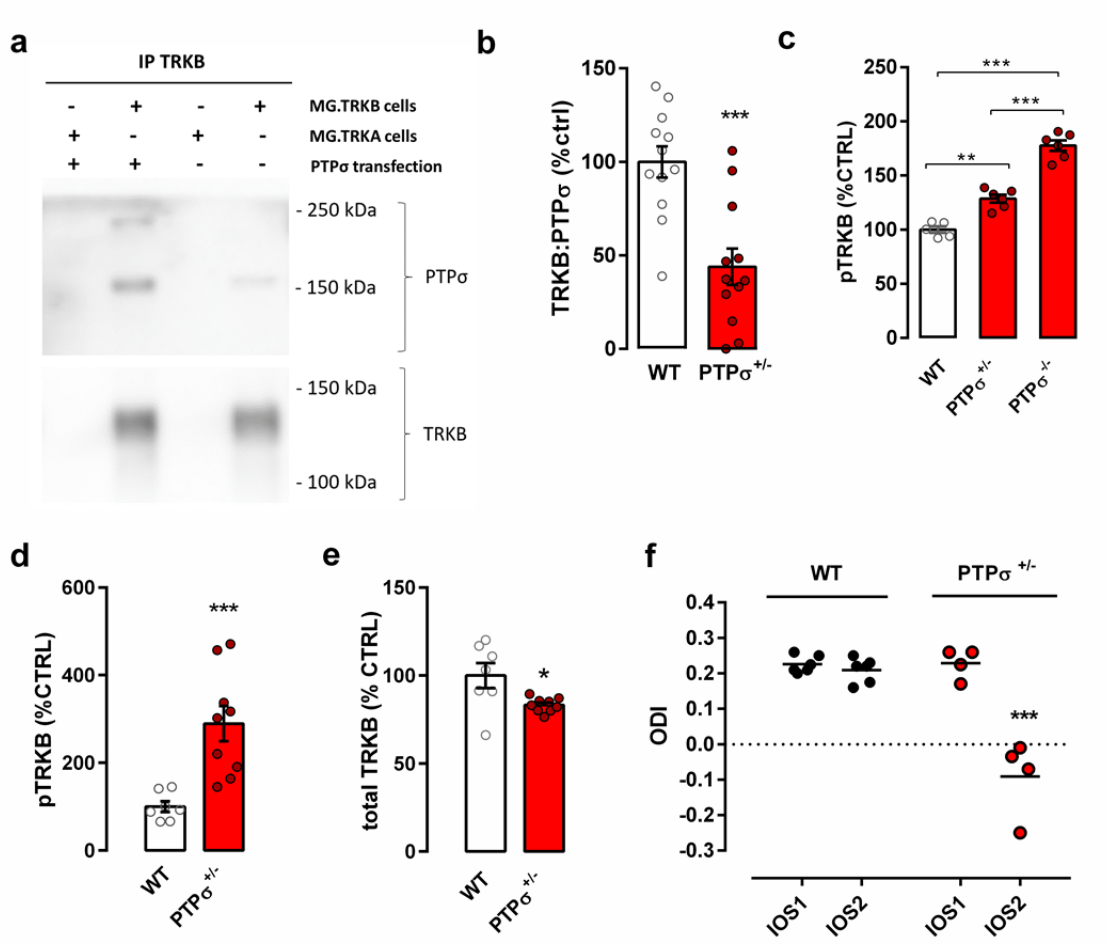


Fig. 2: Deletion of CSPG receptor PTPσ facilitates TRKB phosphorylation and delays closure of critical period in the visual cortex of adult mice. a) PTPσ can be immunoprecipitated with anti-TRKB antibody in samples from TRKB expressing, but not from TRKA expressing cell line and b) TRKB:PTPσ interaction is reduced in embryonic neuronal culture from WT and PTPσ$^{+/-}$ mice. c) TRKB phosphorylation is increased in cortical cultures from PTPσ$^{+/-}$ and PTPσ$^{-/-}$ mice. d) Adult PTPσ$^{+/-}$ mice have increased TRKB phosphorylation and (e) a slight decrease in total TRKB levels in the visual cortex. f) Critical period-like plasticity is still present in the visual cortex of adult PTPσ$^{+/-}$ mice (blue squares) but not in WT littermates (black circles). Columns and bars represent mean±sem, respectively, and scattered points represent individual values. Data was analyzed by two-way (f) or one-way ANOVA (c) followed by Bonferroni´s or Tukey´s post-hoc, respectively; unpaired T test (b) or Mann-Whitney test (d,e). *p<0.05, **p<0.005, ***p<0.0005.

Next, we set out to investigate PTPσ role on TRKB phosphorylation by using mice heterozygous for PTPσ (PTPσ$^{+/-}$). Since transgenic mice quite often develop compensatory mechanisms to counteract genetic deficiency, we wanted to confirm that neurons lacking *PTPRS* allele would present comparable reduction in PTPσ:TRKB protein interaction. We prepared E18 neuronal cultures from cortex of WT or PTPσ$^{+/-}$ littermates, and compared

them after 7 DIV. We observed a reduction of about 50% in the interaction between TRKB and PTPσ (Fig. 2b). Then, we further investigated if PTPσ influences TRKB phosphorylation. We compared cortical pTRKB levels in embryonic cultures from PTPσ$^{-/-}$ and PTPσ$^{+/-}$ mice with those in cultures prepared from their WT littermates. Notably, PTPσ had a clear gene dosage-dependent effect on the basal pTRKB. PTPσ$^{+/-}$ cortical cultures demonstrate increased pTRKB as compared to cultures from their WT littermates, and PTPσ$^{-/-}$ samples demonstrate yet further increased pTRKB significantly differing from both PTPσ$^{+/-}$ and WT samples (Fig. 2c). We also checked the levels of TRKB phosphorylation in samples from the adult visual cortex of mice heterozygous for *PTPRS* gene (PTPσ$^{+/-}$). In line with our *in vitro* data, adult PTPσ$^{+/-}$ mice exhibit increased basal TRKB autophosphorylation in the visual cortex (Fig. 2d), despite having slightly reduced levels of total TRKB (Fig 2e).

Finally, we investigated whether increased TRKB signaling produced by genetic deficiency of PTPσ might render the cortical structures of PTPσ$^{+/-}$ mice susceptible to plastic changes. We tested adult PTPσ$^{+/-}$ mice and their WT littermates in an ocular dominance plasticity paradigm using optical imaging. As expected, monocular deprivation did not induce an ocular dominance shift in adult WT animals; however, it did induce a shift in ocular dominance in PTPσ$^{+/-}$ mice (Fig 2f).

Antidepressant treatment disrupts TRKB:PTPσ interaction

Antidepressants have been demonstrated to induce activation of TRKB receptors in the brain [17,35,36]. Moreover, fluoxetine, a widely used antidepressant, induces ocular dominance plasticity in the adult visual cortex through BDNF-TRKB signaling [21] and reduces percentage of PNNs enwrapping PV+ interneurons in the amygdala and hippocampus, shifting PV^{+} interneurons towards an immature state and reopening brain plasticity [22]. Therefore, we asked whether fluoxetine treatment might have an effect on TRKB:PTPσ interaction, which could potentially establish a link between antidepressant-induced TRKB phosphorylation and PNNs. We cultured rat primary cortical neurons for 7 DIV and challenged them with two different doses of fluoxetine (0.1 and 1 µM) for 30 min. Fluoxetine dose-dependently reduced the interaction between TRKB and PTPσ *in vitro* as measured by ELISA (Fig 3a). Since fluoxetine has been shown to increase TRKB phosphorylation as fast as 30 min after systemic injection [17], we checked if fluoxetine would be able to disrupt TRKB:PTPσ interaction *in vivo,* in a similar timeframe. We treated animals with 30 mg/kg fluoxetine (i.p.) and sacrificed the animals 30 minutes after the injection. Acute fluoxetine treatment significantly reduced TRKB:PTPσ interaction *in vivo* (Fig 3b).

PTPσ is proteolytically processed by cleavage in the juxtamembrane region, leading to shedding of the extracellular domain [27]. To rule out the possibility that TRKB:PTPσ interaction experiments could have been influenced by a potential effect of the treatment upon PTPσ shedding, we investigated the presence of PTPσ extracellular domain in the cell surface after fluoxetine treatment in cortical cells using cell-surface ELISA [34,37]. We found no effect of the drug (Fig 3c). These data indicate that fluoxetine does not affect PTPσ shedding and positioning on cell surface, and supports the idea that the decrease in PTPσ levels in co-IP experiments is a result of decreased interaction with TRKB.

Antidepressants have recently been shown to directly interact with TRKB through the transmembrane region [38]. Moreover, it has been previously suggested that PTPσ interacts with TRKA receptors through its transmembrane region [27]. Therefore, we asked whether disruption in TRKB: PTPσ interaction after fluoxetine treatment is potentially mediated by the antidepressant's binding to the same region of TRKB where interaction with PTPσ takes place. We transfected HEK293T cells with either a wild-type TRKB plasmid or a TRKB plasmid carrying two point mutations in the transmembrane region of TRKB (arginine R427 mutated to alanine, R427A; and tyrosine Y433 mutated to phenylalanine, Y433F); these point mutations disrupt a cholesterol interaction site

in the TRKB transmembrane region critical for antidepressant interaction [39]. R427A/Y433F mutation caused a dramatic decrease in TRKB:PTPσ interaction levels, however, the interaction was not completely lost, suggesting that multiple sites of interaction may exist. Nevertheless, fluoxetine (10 µM) failed to influence TRKB:PTPσ interaction in the cells carrying R427A/Y433F mutated TRKB (Fig. 3d), providing evidence that the site of interaction of PTPσ and fluoxetine with TRKB lies within the transmembrane region of TRKB.

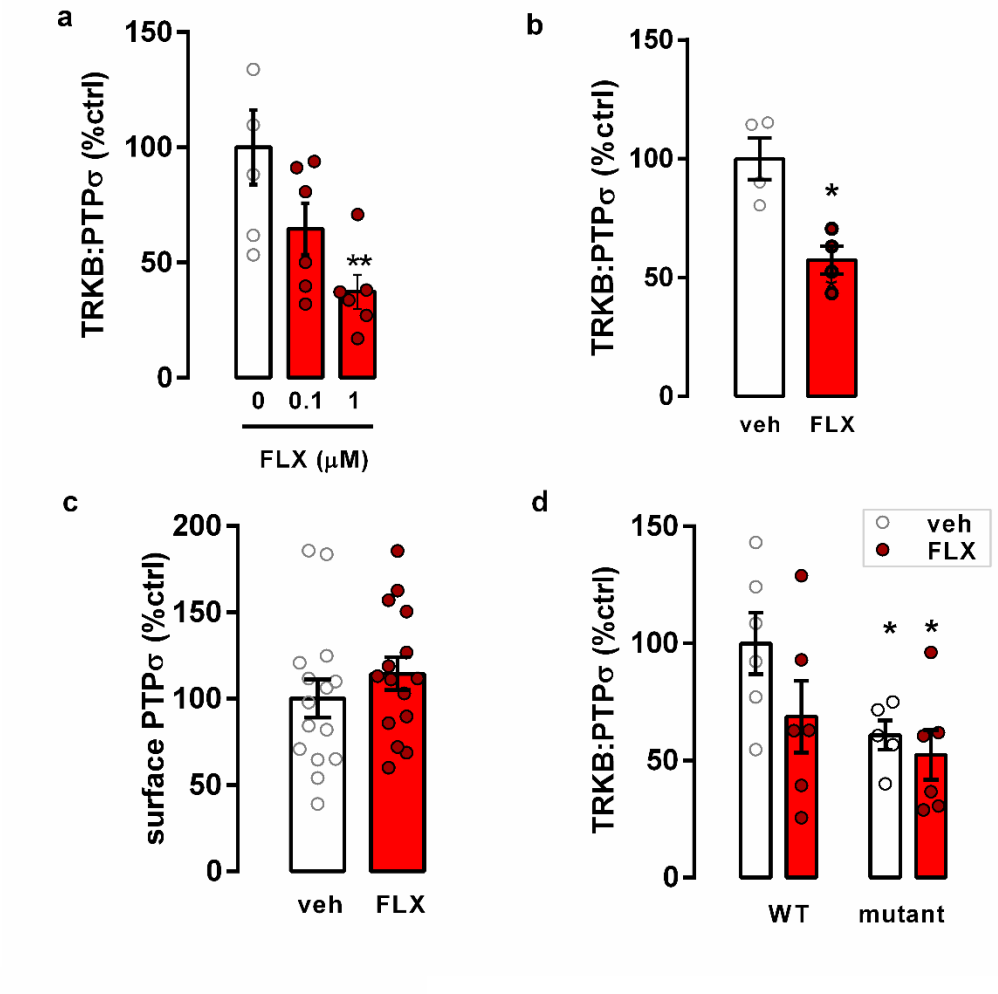


Fig. 3: Fluoxetine disrupts TRKB and PTPσ interaction *in vitro* and *in vivo*. a) Fluoxetine disrupts the interaction between TRKB and PTPσ in 7 DIV cortical neurons, and (b) in the visual cortex of mice systemically treated (30 mg/kg i.p.) 30 min before tissue dissection. c) Fluoxetine did not affect the detection of PTPσ extracellular domain on the cell surface, indicating no effect on PTPσ surface exposure or PTPσ ectodomain shedding. d) Mutation of the TRKB transmembrane domain (R427A/Y433F) disrupts the TRKB:PTPσ interaction and abolished the effects of fluoxetine on it. Columns and bars represent mean±sem, respectively. Data was analyzed by one-way ANOVA followed by Bonferroni´s (a), two-way ANOVA (d), or unpaired t test (b and c). *p<0.05, **p<0.005.

Discussion

In the current study, we have investigated a possible convergence of the two plasticity-inducing methods, chABC and antidepressant treatment, on the same molecular pathway involving reduced dephosphorylation of TRKB by PTPσ within PV^+ interneurons. Perineuronal nets, extracellular structures rich in CSPGs, are associated with a reduction of plasticity in the adult brain. Maturation of PNNs coincides with the closure of the critical period [29,40], and digestion of PNNs has been shown to promote plasticity and re-open critical periods for certain functions in the adult nervous system [29]. Local injections of chondroitinase ABC promote axonal regeneration and functional recovery after spinal cord injury [11], restore ocular dominance plasticity in the visual cortex [10], and promote extinction of fear memories in rodents [12]. Perineuronal nets preferentially enwrap PV^+ interneurons that synchronize oscillatory activity of brain networks and mediate neuronal plasticity and learning. Our experiments demonstrate that the effects of chABC depend on TRKB signaling in PV^+ interneurons, since genetic deficiency of TRKB in PV^+ cells abrogates the ability of chondroitinase ABC to induce plasticity in the visual cortex of adult mice. Moreover, we have shown that chABC treatment increases pTRKB, which promotes TRKB signaling, while treatment with CSPG aggrecan decreases phosphorylation of TRKB induced by BDNF.

Chondroitin sulfate proteoglycans, major components of perineuronal nets, have been shown to exert inhibitory action on plasticity through protein tyrosine phosphatase receptor type S [24]. PTPσ has been demonstrated to be critical for the inhibitory effects of CSPGs on plasticity and regeneration since CSPGs exert little inhibitory effect on axonal outgrowth of neurons prepared from $PTP\sigma^{-/-}$ mice as compared to WT [24]. Interestingly, PTPσ has been shown to interact with and dephosphorylate all three TRK receptors and inhibit their activation even in the presence of their cognate neurotrophins [27]. Faux et al. provided evidence that PTPσ forms stable complexes with TRKA and TRKC and only weakly interacts with TRKB. However, subsequent research has shown that PTPσ co-immunoprecipitates with TRKB in samples from cortical neurons and mediates abrogation of BDNF-induced dendritic spine formation by CSPGs [28]. We have now demonstrated that PTPσ dephosphorylates TRKB in *vitro* and in *vivo* based on increased phosphorylation of TRKB in cultures prepared from PTPσ KO homozygous or heterozygous mice ($PTP\sigma^{-/-}$ and $PTP\sigma^{+/-}$) and in samples from the visual cortex of $PTP\sigma^{+/-}$ mice. Moreover, we observed that genetic deficiency of PTPσ delays the closure of critical period-like plasticity in the adult visual cortex, an effect that we demonstrated to be dependent on TRKB in PV^+ neurons.

TRKB signaling is known to be critical for activation of plasticity by antidepressant drug treatment and for the behavioral consequences of it [17–20]. Moreover, our group has recently demonstrated that TRKB activation in parvalbumin-positive interneurons is sufficient for the induction of juvenile-like plasticity in the adult brain and necessary for the plasticity-inducing effects of fluoxetine [23]. We now demonstrate that the antidepressant fluoxetine induces disruption of interaction between TRKB and PTPσ, releasing TRKB from the suppressive activity of the phosphatase and promoting its activation. These data suggest that reduced dephosporylation of TRKB by PTPσ is at least one mechanism through which antidepressant treatment promotes plasticity.

Antidepressants have recently been shown to directly interact with TRKB through its transmembrane domain [38]. Our data now show that a mutation that inhibits antidepressant binding to the transmembrane region of TRKB partially disrupts its interaction with PTPσ, and that fluoxetine treatment has no further additive effect on TRKB:PTPσ interaction in TRKB mutant cells. These data suggest that TRKB and PTPσ interact in the transmembrane region of TRKB, and that binding of fluoxetine to TRKB disrupts TRKB: PTPσ interaction.

Our findings suggest a general mechanism responsible for the opening of critical period-like plasticity in the adult brain by chondroitinase ABC and antidepressant treatment. We propose that both methods converge on

the same pathway involving reduced inhibitory interaction between TRKB and PTPσ in PV^+ neurons, which releases TRKB and promotes its autophosphorylation. Chondroitinase treatment downregulates CSPGs-mediated activation of PTPσ, which allows for enhanced signaling of TRKB. Antidepressants, on the other hand, disrupt the interaction between TRKB and PTPσ in the membrane, promoting TRKB activation. Taken together, our data reveal that interaction between TRKB and PTPσ in PV^+ interneurons is a critical regulator of plasticity in the adult cortex. There are a number of other methods known to reactivate juvenile-like plasticity in the adult cortex, such as enriched environment [41] and cross-modal manipulation of sensory functions [42]. It will be interesting to investigate whether induction of plasticity by these means recruits the same molecular pathways involving enhanced TRKB activation in PV^+ interneurons.

Materials and Methods.

Animals. Balb/c and C57BL/6J mice heterozygous for *PTPRS* gene ($PTP\sigma^{+/-}$ mice; homozygous $PTP\sigma^{-/-}$ mice only rarely survive until adulthood) and their wild-type littermates and C57BL/6J mice heterozygous for *TRKB* gene in parvalbumin-expressing interneurons (PV-$TRKB^{+/-}$; homozygous PV-$TRKB^{-/-}$ mice suffer from a vestibular phenotype and cannot be reared to adulthood) were used in the experiments. Balb/c $PTP\sigma^{+/-}$ mice were originally developed by Michel Tremblay's lab (McGill University, Canada) [43] and kindly donated to us by Heikki Rauvala (University of Helsinki, Finland). Balb/c $PTP\sigma^{+/-}$ mice, 10 weeks old, were used for brain sample collection and pTRKB level assessment by ELISA [33]. For optical imaging experiments, the balb/c mouse line was rederived to a C57BL/6J background, and N4 generation of the offspring was used for testing. The mice were 2 months old at the beginning of the experiments. PV-$TRKB^{+/-}$ ($TRKB^{flx/wt}$, $PV^{cre/wt}$) mice were generated by mating heterozygous floxed TRKB mice ($TRKB^{flx/wt}$) [30] and homozygous PV-specific Cre line ($PV^{cre/cre}$) (Pvalb-IRES-Cre, JAX: 008069, Jackson laboratory) [44]. PV heterozygous ($TRKB^{wt/wt}$, $PV^{cre/wt}$) littermates were used as a control group. The mice were 4 months old at the beginning of the experiments. The mice were kept under standard laboratory conditions with 12-hour light/dark cycle (lights on at 6:00 am) and access to food and water *ad libitum*. All the procedures involving animals were done in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals guidelines and were approved by the experimental Animal Ethical Committee of Southern Finland (ESAVI/10300/04.10.07/2016, ESAVI 38503/2019).

Antibodies. Anti-phospho-TRKA (Tyr490)/TRKB (Tyr516) (C35G9) rabbit monoclonal antibody (mAb) was purchased from Cell Signaling Technology, USA (#4619). Anti-TRKB goat polyclonal antibody (pAb) was bought from R&D Systems, USA (#AF1494). Anti-phosphotyrosine mouse monoclonal antibody (clone PY20) was purchased from Bio-Rad, USA (#MCA2472). Anti-PTPσ (SS-8) mAb was purchased from Santa Cruz Biotechnology, USA (SC-100419). Anti-β-Actin (AC-15) mouse mAb was acquired from Sigma Aldrich (#A1978). Secondary HRP-conjugated antibodies were purchased from Bio-Rad, USA (goat anti-rabbit #1705046 and goat anti-mouse #1705047) and from Invitrogen, USA (rabbit anti-goat #611620).

Optical imaging of PV-$TRKB^{+/-}$ mice. Four-month-old mice were used for the experiments. During week 1, the animals underwent transparent skull surgery. After 7 days, during week 2, the animals underwent the first session of the optical imaging under isoflurane anesthesia (IOS1). During week 3, the animals were injected with 50 mU of chABC in PBS or PBS into the binocular area of the visual cortex and were subjected to monocular deprivation (MD) for 7 days. During week 4, the eyes were opened, and IOS2 immediately took place (for details, see Supplementary material and [32]).

Optical imaging of $PTP\sigma^{+/-}$ mice. Two-month-old mice were used for experiments. During week 1, the animals underwent transparent skull surgery. After 7 days, the animals underwent the first session of the optical imaging

(IOS1) followed by MD for 3.5 days [45]. On day 4 of the monocular deprivation, the eyes were opened, and IOS2 took place (for details, see Supplementary material and [32]).

Statistical analysis: Parametric tests were preferentially used to gain statistical power. Exceptions were made whenever the data presented lacked homoscedasticity or when variables were discrete, in which cases non-parametric tests were chosen. The statistical tests used in each particular experiment are described in the legend of figures, and statistical values are provided in the supplementary material. Differences were considered statistically significant when $p<0.05$. Statistical analysis and plots were made in GraphPad Prism 6 software. Data are presented as mean± SEM.

Detailed information on materials and methods is provided in the Supplementary material**.**

Author contributions: Conceptualization: CB and EC; Investigation: AL, PC, MF, MV, FW, AS, HA, JU, CB; Formal Analysis: AL, PC, MF, MV, AS, CB; Writing – Original Draft: AL, CB and EC; Writing – Review & Editing: AL, CB, EC, MF, MV, FW, AS, HA, PC, JU; Visualization: CB and AL; Supervision: CB and EC; Funding Acquisition: EC.

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