Research Article

# Neuronal protein-tyrosine phosphatase 1B hinders sensory-motor functional recovery and causes affective disorders in two different focal ischemic stroke models

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## Abstract

Ischemic brain injury causes neuronal death and inflammation. Inflammation activates protein-tyrosine phosphatase 1B (PTP1B). Here, we tested the significance of PTP1B activation in glutamatergic projection neurons on functional recovery in two models of stroke: by photothrombosis, focal ischemic lesions were induced in the sensorimotor cortex (SM stroke) or in the peri-prefrontal cortex (peri-PFC stroke). Elevated PTP1B expression was detected at 4 days and up to 6 weeks after stroke. While ablation of PTP1B in neurons of neuronal knockout (NKO) mice had no effect on the volume or resorption of ischemic lesions, markedly different effects on functional recovery were observed. SM stroke caused severe sensory and motor deficits (adhesive removal test) in wild type and NKO mice at 4 days, but NKO mice showed drastically improved sensory and motor functional recovery at 8 days. In addition, peri-PFC stroke caused anxiety-like behaviors (elevated plus maze and open field tests), and depression-like behaviors (forced swimming and tail suspension tests) in wild type mice 9 and 28 days after stroke, respectively, with minimal effect on sensory and motor function. Peri-PFC stroke-induced affective disorders were associated with fewer active ($FosB^+$) neurons in the PFC and nucleus accumbens but more $FosB^+$ neurons in the basolateral amygdala, compared to sham-operated mice. In contrast, mice with neuronal ablation of PTP1B were protected from anxiety-like and depression-like behaviors and showed no change in $FosB^+$ neurons after peri-PFC stroke. Taken together, our study identifies neuronal PTP1B as a key component that hinders sensory and motor functional recovery and also contributes to the development of anxiety-like and depression-like behaviors after stroke. Thus, PTP1B may represent a novel therapeutic target to improve stroke recovery. All procedures for animal use were approved by the Animal Care and Use Committee of the University of Ottawa Animal Care and Veterinary Service (protocol 1806) on July 27, 2018.

**Key Words:** adhesive removal test; anxiety; depression; elevated plus maze; forced swimming test; Iba1; interleukin-1β; microglia; open field test; tail suspension test; tumor necrosis factor-α

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## Introduction

Stroke can cause debilitating sensory, motor, cognitive and communication deficits. Furthermore, in patients surviving stroke, mood disorders such as anxiety and depression not only affect patients' quality of life but also directly impact on their motivation and adherence to physical rehab to improve functional recovery after stroke (Chollet et al., 2011; Mikami et al., 2011). Anxiety and depression after stroke may arise from despair and inability to cope with daily tasks, or as a direct consequence of injury to the limbic system. Even after a transient ischemic attack (TIA) that leaves patients free of sensory and motor deficits, nearly 30% of TIA patients develop posttraumatic stress disorder (anxiety and depression) (Kiphuth et al., 2014). Moreover, given that as many as 8 out of every 10 persons in an aged population (~62 years old) has had a silent stroke (Das et al., 2008), a form of stroke that can only be detected by MRI imaging, and that silent stroke increases the risk of depression 2–3 fold (Wu et al., 2014), the prevalence of post-stroke anxiety and depression is likely underestimated.

The location of ischemic brain injury greatly influences the nature of the functional deficits: Lesions at the sensorimotor (SM) cortex cause severe sensory and motor deficits, whereas

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lesions to the left prefrontal cortex (PFC) can produce anxiety and depression with minimal deficits in sensory and motor function in humans and mice (Robinson et al., 1984; Terroni et al., 2011; Kronenberg et al., 2012; Vahid-Ansari et al., 2016). Focal ischemic lesions to the motor cortex induced by photothrombosis were reported to cause severe motor deficits peaking on the 4$^{th}$ day with a gradual but partial recovery on the 6$^{th}$ day (Li et al., 2014a). The extensive rewiring of the motor cortex in the area surrounding ischemic lesions correlates to this recovery [elegantly reviewed in (Murphy and Corbett, 2009)].

The PFC is an important limbic region that limits anxiety and depression (Bremner, 1999; Drevets, 2001; Keedwell et al., 2005; Mayberg et al., 2005; Feder et al., 2009) by suppressing neuronal activity in the basolateral amygdala (BLA) and by increasing activity in the nucleus accumbens (NAc), respectively (Vialou et al., 2014). Positron emission tomography (PET) and single-photon emission computed tomography imaging studies reveal reduced activity in the PFC in most types of depression, including stroke-induced depression (Mayberg, 1994), that can be alleviated by deep brain stimulation of the PFC (Mayberg et al., 2005; Nahas et al., 2010), but this approach is invasive.

Selective serotonin reuptake inhibitors (SSRI) are currently used for post-stroke depression. However, 30–50% of patients with depression fail to respond to SSRI (Krishnan and Nestler, 2008), and this may reflect disease mechanisms of depression, such as increased inflammation, that are not targeted by SSRI (Hodes et al., 2015). Moreover, a Danish study of ~6000 stroke patients found a significant increase in mortality among those taking SSRI (Mortensen et al., 2013). A more recent and larger study (> 16,000 patients) reported that patients receiving antidepressant therapies targeting the norepinephrine or serotonin systems (tricyclic antidepressants, SSRI or monoamine oxidase inhibitors) have an 84% increase in the risk of stroke recurrence (Juang et al., 2015). Importantly, the SSRI Fluoxetine had no effect on motor recovery after stroke, although it did improve mood disorders while increasing the frequency of bone fractures (Collaboration, 2019). Identifying new targets and safe alternative methods to alleviate post-stroke anxiety and depression that could also promote motor recovery from stroke are needed.

Stroke causes a profound and long-lasting inflammatory response (Mena et al., 2004; Gerhard et al., 2005; Gulyas et al., 2012). Inflammation increases expression of the tyrosine phosphatase PTP1B in neurons (Zabolotny et al., 2008; Zhu et al., 2015; Song et al., 2016; Tsunekawa et al., 2017), but the significance of PTP1B to stroke outcome and recovery has not been addressed. Here, we tested the hypothesis that neuronal PTP1B activation would affect motor recovery from stroke and contribute to the manifestation of affective deficits after limbic stroke.

## Materials and Methods

### Animals

Cam2αCre/PTP1Bflox mice that ablate PTP1B in glutamatergic projection neurons (PTP1B NKO) (Ricke et al., 2020) were generated by breeding PTP1Bflox mice (Bence et al., 2006) with the Camk2αCre mice (Casanova et al., 2001) on the C57BL6 background. Mice were fed with regular chow, and randomly assigned to sham, SM stroke or peri-PFC stroke groups. All procedures for animal use were approved by the Animal Care and Use Committee of the University of Ottawa Animal Care and Veterinary Service (protocol 1806) on July 27, 2018 and were in accordance with institutional guidelines and those of the Canadian Council on Animal Care.

### Photothrombotic SM and peri-PFC stroke surgery

Focal stroke was induced on the left side by photothrombosis using light-sensitive dye that triggers clots in the blood vessels, as described previously (Watson et al., 1985). 2-Month-old mice were anesthetized with isoflurane gas, and placed under a stereotaxic frame. A midline incision of the scalp was made to expose a 1 mm × 1 mm region of the left skull. Five minutes after administration of the light-sensitive dye Rose Bengal (Sigma-Aldrich, Oakville, ON, Canada) (10 mg/mL in PBS, made fresh by vortexing and filtered, 100 mg/kg, i.p.), a collimated green laser (532 nm, 20 mW, Beta Electronics, Columbus, OH, USA) was placed 2 cm above the skull at the following coordinates: anteroposterior 0.7 mm, mediolateral 2 mm (for SM-stroke) or anteroposterior: 2 mm, mediolateral: 0.6 mm (for peri-PFC stroke) relative to the Bregma, according to the atlas (Franklin and Paxinos, 2008), to illuminate the skull for 10 minutes to initiate photothrombosis. During the procedure and post-operative recovery, the body temperature was maintained at 37.5 ± 0.5°C with a heated pad.

### Infarct volume measurement

Areas of exclusion of Cresyl violet staining of Nissl bodies in living neurons (Schock et al., 2008) were quantified from 10 coronal sections (20 µm thick), sampled every 10 sections over a 2 mm distance overlapping the area of infarction, as described previously (Cruz et al., 2017). Infarct volumes were calculated by stacking infarct areas of exclusion in serial sections using the ImageJ software (US National Institutes of Health, Bethesda, MD, USA). Lesion volumes are reported in mm$^3$. Of note, our previous study showed that there is little residual edema 4 days after photothrombosis, obviating the need for normalization of lesions to total brain volume (Cruz et al., 2017). Investigators were blinded to genotype of mice.

### Behavior tests

Sensory and motor functions were assessed by an adhesive-removal test, as we described previously (Cruz et al., 2017). Elevated plus maze and open field tests were used as an index of anxiety and were performed as we described (Qin et al., 2015b). Immobility time during forced swim test and tail suspension test were used as an index of depression (Castagne et al., 2011). Investigators were blinded to genotype of mice.

#### *Adhesive-removal test*

The adhesive-removal test was performed 7 days prior to, and 4, 8 or 14 days after SM-stroke and 4 days after peri-PFC stroke (**Figure 1A**). An adhesive tape is placed on the palms of mice and the times to detect and to remove the tape are measured as an index of sensory and motor function, respectively. Mice were removed from their home cage and were transferred to a new clean cage placed in a testing room for 30 minutes to habituate. For testing, an adhesive tape that covers the hairless part of the paw was pressed gently into the left and right forelimb paw of a restrained mouse. The mouse was then placed in the testing cage and the latency required for paw-to-mouth contact and tape removal was recorded, with a maximum of 2 minutes per trial period. Data collected includes left and right contact time and left and right removal time.

#### *Elevated plus maze test*

The elevated plus maze test was performed on day 9 after photothrombosis (**Figure 2A**). The elevated 4-arm maze has two open arms (6 cm × 35 cm) and two closed arms (6 cm × 35 cm × 20 cm) that extend from a central platform (6 cm × 6 cm) elevated approximately 75 cm above the floor. Mice were placed individually in the center of the maze facing the open arms and allowed to explore the maze for 10 minutes. The session was video recorded and the results were analyzed with a computerized tracking system (Ethovision 8 software, Noldus, Wageningen, Netherlands). Parameters measured included number of open arm entries as well as the time spent in open arms.

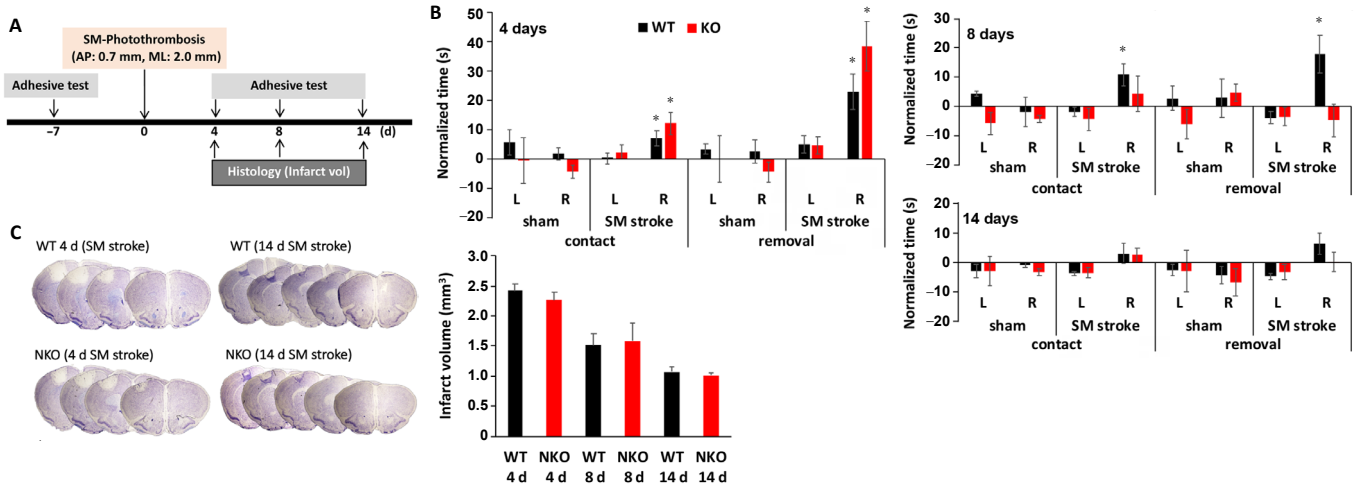


**Figure 1** | **Sensory and motor function recover faster in protein-tyrosine phosphatase 1B knockout (PTP1BNKO) mice after photothrombosis-induced lesion at the sensorimotor cortex (SM stroke).**
(A) Timeline of adhesive removal tests carried out at 4, 8, 14 days after photothrombosis-induced SM stroke. (B) PTP1BNKO (NKO) mice show a markedly improved performance of the adhesive removal test between 4–8 days post-SM-stroke compared to littermate controls (WT). The latency to contact and removal of adhesive tape are associated with sensory and motor functional deficits, respectively. The times required to perform a task after stroke is subtracted from the time required prior to stroke. $n$ = 14 WT, 9 NKO each group. *$P$ < 0.05, *vs.* contralateral side (two-way analysis of variance followed by Tukey's *post hoc* test). (C) Cresyl violet staining comparing the infarct area (pale regions of left hemisphere) after SM stroke. $n$ = 9–11 each group.

### *Open field test*

The open field test was performed on day 37 after photothrombosis (**Figure 2A**). Mice were placed in an open field box (45 cm × 45 cm × 45 cm; MED Associates, Fairfax, VT, USA) illuminated with 300 lx intensity to enhance anxiety triggered by exposure to a novel large and bare open arena. Mice were videotaped for 10 minutes and the time they spent at the center (25 cm × 25 cm) and at the periphery of the chamber (10 cm × 10 cm corners) were analyzed using video tracking software Noldus (Ethovision).

### *Tail suspension test*

The tail suspension test measures the stress coping response as an index of depression (Castagne et al., 2011) on day 42 after photothrombosis (**Figure 2A**). Mice were suspended by taping the tail to a metal bar in a tail suspension box (MED Associates) for 6 minutes and an automated detection device (ENV-505TS Load-Cell Amplifier, MED Associates) recorded and identified immobility times.

### *Forced swim test*

The Forced swim test was also used to assess depression-like behavior (Castagne et al., 2011) on day 28 after photothrombosis (**Figure 2A**) by subjecting Mice to an inescapable swim stress for 6 minutes. Mice were placed in 4 L of water (24°C) in a clear plastic cylinder (22cm diameter, 37 cm deep) and videotaped from the side of the cylinder under red lighting. The duration of immobility time was determined using Ethovision XT automated video-tracking software (MED Associates).

### Quantitative polymerase chain reaction

Total RNA from brain tissue (prefrontal cortex, amygdala, and nucleus accumbens) was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Canada) (Zhou et al., 2012). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was conducted as described previously (Chen et al., 2015), and the results were normalized to β-actin. Primers used for qPCR: TNF-α: (F) 5′-CCA CCA CGC TCT TCT GTC TAC-3′, (R) 5′-AGG GTC TGG GCC ATA GAA CT-3′. IL-1β: (F) 5′-CAG GCT CCG AGA TGA ACA A-3′, (R) 5′-CCC AAG GCC ACA GGT ATT T-3′. Actin: (F) 5′-CCT TCT GAC CCA TTC CCA CC, reverse 5′-GCT TCT TTG CAG CTC CTT CG-3′. PTP1B: (F) 5′-TGG CTG ACA CCT GCC TCT TA-3′, (R) 5′-CCA CTG ATC CTG CAC TGA CG-3′.

### Immunoblotting and immunofluorescence

Protein extraction of the left and right hemispheres and immunoblot analysis were performed as previously described (Chen et al., 2007b; Gomez-Smith et al., 2010). For immunoblot analysis, antibodies and dilutions were as follows: PTP1B (Abcam, Toronto, ON, Canada, Cat# ab245984, rabbit monoclonal antibody, 1:1000 dilution), β-actin (Sigma-Aldrich, Canada, Cat# A5441, mouse monoclonal antibody, 1:1000 dilution) and appropriate peroxidase-conjugated secondary antibodies (ThermoFisher Scientific, Nepean, ON, Canada, Cat# G-21040: goat-anti mouse IgG, G-21234, goat-anti rabbit IgG, both at 1:10,000 dilution) were revealed by chemiluminescence (GE Healthcare, Mississauga, ON, Canada). PTP1B protein levels were quantified using ImageJ software. Three days after the last behavior test, mice were sacrificed and whole brain cryostat sections (20 µm) were subjected to immunofluorescence, as described (Chen et al., 2007a; Zaman et al., 2014). Immunofluorescence images were acquired on a Zeiss Z1 fluorescent microscope (Zeiss, North York, ON, Canada). Primary antibodies used and their dilutions are: ionized calcium binding adaptor molecule 1 (Iba1) (WAKO Chemicals USA, Richmond, Virginia, Cat# 019-19741, rabbit polyclonal, 1:500 dilution) to label microglia (Cruz et al., 2018), and FosB (Abcam, Canada, Cat# 11959, mouse monoclonal, 1:500 dilution) to label actively firing neurons (Renthal et al., 2008; Vialou et al., 2010). Cy2-, Cy3-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA, Cat# 715-225-150: cy2 conjugated-donkey anti-mouse, #711-165-152: cy3-conjugated-donkey anti-rabbit, both at 1:1000 dilution) were used. For immunofluorescence images, three independent fields at 20× magnification from six sections were imaged and $FosB^+$ cells counted using ImageJ.

### Statistical analysis

All results are presented as the mean ± SEM. For between-group comparisons of fold changes (qPCR), values were normalized by log transformation, whereas percentages were normalized by arcsin transformation. Main effects of genotype and photothrombosis were analyzed by two-way analysis of variance and *post hoc* pairwise comparisons used Tukey's test. Differences in means were considered significant at $P$ < 0.05.

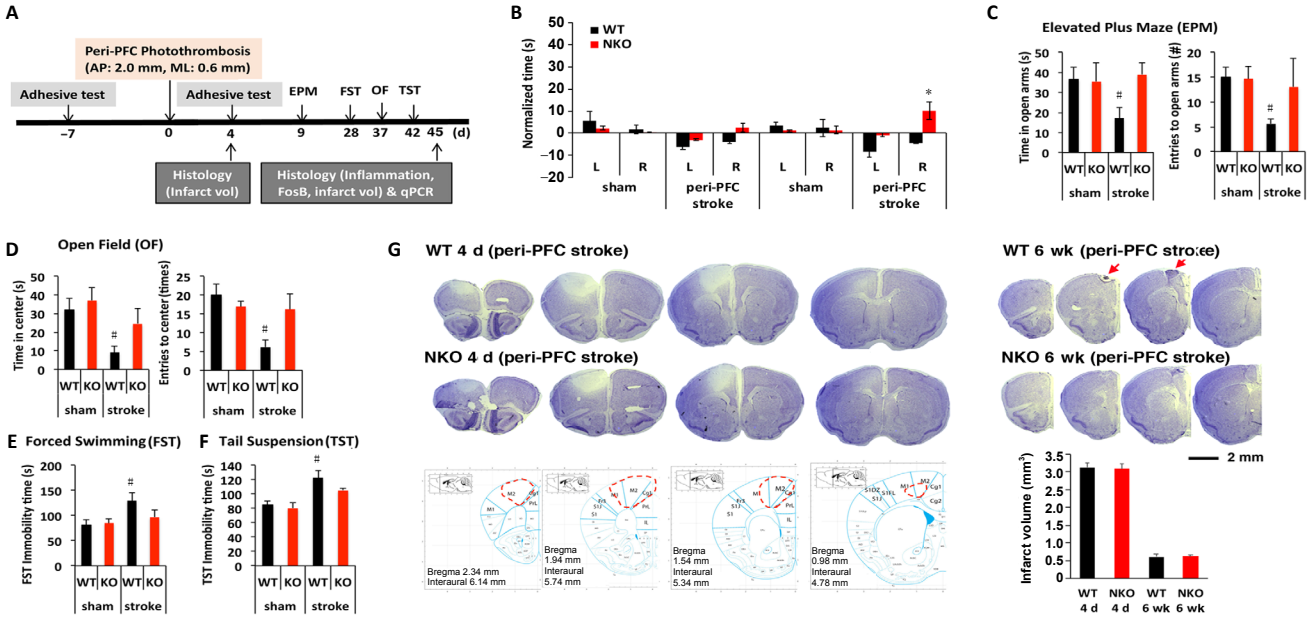


**Figure 2** | **Peri-PFC stroke has minimal impact on sensory-motor function, but causes anxiety and depression in wild type (WT) but not protein-tyrosine phosphatase 1B knockout (PTP1BNKO) mice.**
(A) Timeline of adhesive removal and affective behavior tests carried out after photothrombosis-induced peri-PFC stroke. (B) Adhesive removal test showed no significant sensory and only modest motor deficits 4 days after peri-PFC stroke (compared to SM-stroke in Figure 1B). Peri-PFC stroke caused anxiety, measured by the elevated plus maze (C) and open field test (D), and depression, measured by increased immobility time in the forced swimming test (E) and tail suspension test (F) in littermate controls (WT) but not in PTP1BNKO (NKO) mice. $n$ = 10–15 mice/group. *$P$ < 0.05, *vs.* contralateral side; #$P$ < 0.05, *vs.* WT sham mice (two-way analysis of variance followed by Tukey's *post hoc* test). (G) Cresyl violet staining showed a similar-sized peri-PFC infarction (pale region or red arrows) in NKO mice as in WT at 4 days that regressed to the same extent by 6 weeks. Representative brain map diagrams are shown under each section. The dashed red lines outline the infarct areas. $n$ = 6 mice/group. PFC: Peri-prefrontal cortex.

## Results

### PTP1BNKO mice have faster functional recovery after sensorimotor stroke

PTP1B expression was elevated in both ipsilateral (left) and contralateral (right) sides 4 days after photothrombosis-induced ischemic lesions in the left sensorimotor cortex (SM-stroke) (**Figure 3**). To determine the significance of neuronal PTP1B elevation on functional deficits, mice with neuronal-specific ablation of PTP1B (NKO mice) were generated by crossing PTP1Bflox mice (Bence et al., 2006) with Camk2αCre mice (Casanova et al., 2001). Unlike the previously described Nestin-Cre/PTP1Bflox mice where PTP1B is ablated in all neuroprogenitors (Bence et al., 2006), our NKO mice ablated PTP1B in glutamatergic projection neurons (Ricke et al., 2020) and showed no difference in metabolic properties compared to their littermate controls (data not shown).

NKO mice and their littermate controls were subjected to adhesive removal test at various times after SM-stroke. SM-stroke caused sensory and motor deficits (Cruz et al., 2017) as revealed by the increased contact times (the time required to detect the tape) and removal times (the time required to remove the tape) in the adhesive tape removal test, respectively (**Figure 1B**). Mice with ablation of PTP1B in projection neurons (NKO) showed similar sensory and motor deficits as littermate controls 4 days after SM-stroke, but showed a markedly improved functional recovery at 8 days (**Figure 1B**). Intriguingly, no difference in infarction volumes was detected after stroke between NKO and wild type littermate controls (**Figure 1C**). These results indicate that stroke-induced activation of PTP1B in neurons delays sensory and motor functional recovery.

### PTP1BNKO mice are protected from anxiety and depression-like behaviors after peri-PFC stroke

To investigate the contribution of neuronal PTP1B in post-stroke affective disorders, focal ischemic lesions were targeted to the left peri-prefrontal cortex (peri-PFC). In wild type mice, peri-PFC stroke did not cause measurable

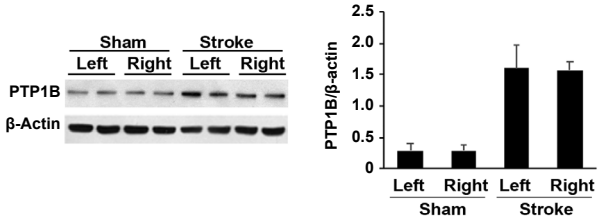


**Figure 3** | **PTP1B expression is elevated after stroke.**
Immunoblots show elevated PTP1B expression in peri-infarct area of both left and right hemispheres 4 days after focal lesion of the left somatosensory cortex. PTP1B (~37 kDa) levels were normalized to β-actin (~42 kDa) for quantification. $n$ = 3–4 mice per group. PTP1B: Protein-tyrosine phosphatase 1B.

sensory or motor deficits 4 days after stroke (**Figure 2B**), in marked contrast to what we observed for SM stroke (**Figure 1B**). Despite the lack of sensory or motor deficits, peri-PFC stroke caused anxiety-like behaviors detected as early as 9 days after stroke in the elevated plus maze (**Figure 2C**) and also in the open field test 1 month after stroke (**Figure 2D**). A depression-like phenotype was also detected in the forced swimming test at 4 weeks (**Figure 2E**) and the tail suspension tests at 6 weeks (**Figure 2F**) after peri-PFC stroke.

As for NKO mice, a slight motor deficit was detected 4 days after peri-PFC stroke (**Figure 2B**), despite a similar infarction volume as in wild type mice at 4 days (**Figure 2G**). It should be noted that this motor deficit is much smaller than observed 4 days after SM stroke (**Figure 1B**), likely reflecting the site of photothrombosis. Remarkably, in contrast to littermate controls, NKO mice did not develop anxiety or depression-like behaviors after peri-PFC stroke (**Figure 2C–F**). We were surprised to note that infarct volumes had regressed by ~80% in both wild type and NKO mice by 6 weeks after peri-PFC stroke (**Figure 2G**), yet anxiety and depression-like behaviors were evident only in wild type but not NKO mice.

We asked whether neuronal PTP1B ablation affects the

inflammatory response after peri-PFC stroke to account for the absence of anxiety and depression in NKO mice. Immunostaining revealed sustained microglial activation even 6 weeks after peri-PFC stroke in both wild type (**Figures 4A** and **5**) and NKO mice (**Figure 5**). Moreover, the mRNAs for inflammatory cytokines TNF-α (**Figure 4B**) and IL-1β (**Figure 4C**) were similarly elevated in NKO and wild type mice, on the lesioned ipsilateral as well as the contralateral prefrontal cortex, amygdala and nucleus accumbens. Therefore, neuronal ablation of PTP1B did not affect the inflammatory response after peri-PFC stroke, at least for these 2 cytokines. The fact that the inflammation-responsive gene PTP1B (Zabolotny et al., 2008; Zhu et al., 2015; Song et al., 2016) remains elevated in wild type mice 6 weeks after peri-PFC stroke (**Figure 4D**) and is associated with persistent anxiety/depression and that these affective disorders did not appear in NKO mice strongly supports the notion that PTP1B activation in neurons is the major driving force for the development of anxiety and depression-like behaviors after stroke.

### Altered neuronal activity after peri-PFC-stroke

We next examined whether altered neuronal activity might correlate with the appearance of anxiety and depression-like behaviors after peri-PFC stroke. FosB expression, an index of persistent neuronal activation (Renthal et al., 2008; Vialou et al., 2010), was compared in various brain regions implicated in affective disorders. In wild type mice 6 weeks after peri-PFC-stroke, fewer $FosB^+$ neurons were counted in the PFC (**Figure 5A** and **D**) and NAc (**Figure 5B** and **D**) while more $FosB^+$ neurons were counted in the BLA (**Figure 5C** and **D**), compared to sham-operated wild type mice. This peri-PFC stroke-induced change in the number of $FosB^+$ neurons was not observed in NKO mice, nor was it correlated with global microglia activation (**Figure 5A–D**). Of note, similar numbers of $Iba1^+$ microglia were detected in WT and NKO mice after stroke. Thus, our data suggest that neuronal PTP1B activation reduces activity in PFC and NAc neurons, but elevates activity in BLA neurons after peri-PFC stroke. Our data further support the notion that reduced PFC and NAc neuronal activity together with increased BLA activity might contribute to anxiety and depression (Anand et al., 2005; Kim et al., 2011; Tan et al., 2011; Sripada et al., 2012; Admon et al., 2013; Goff et al., 2013; Shonesy et al., 2014; Vialou et al., 2014) after stroke.

## Discussion

The present study reports several important findings: First, neuronal PTP1B ablation does not affect infarct volumes. Second, ablation of PTP1B in neurons improves sensory and motor recovery after SM stroke. Third, ischemic injury to the limbic system by peri-PFC photothrombosis, while having a minimal effect on sensory/motor function, causes anxiety and depression-like behaviors. Finally, anxiety and depression after limbic ischemic injury can be prevented by neuronal ablation of PTP1B.

Consistent with prior histological (Mena et al., 2004) and positron emission tomography imaging (Gerhard et al., 2005; Gulyas et al., 2012) studies in humans that found inflammatory microglia/macrophages persist for several months after ischemic brain injury, we found persistent microglial activation with elevated IL-1β and TNF-α inflammatory cytokine mRNAs even 6 weeks after photothrombosis in wild type as well as in NKO mice. Of note, it was reported that photothrombosis-induced inflammation impairs sensory learning whereas anti-inflammatory drugs could rescue sensory deficits (Greifzu et al., 2011). Along these lines, patients suffering chronic sensory deficits long after stroke showed improvement after spinal anti-TNF-α treatment (Tobinick, 2011), indicating that inflammation after stroke impairs sensory function.

After ischemic injury, the surviving cortex undergoes profound rewiring, forming new synapses and this process is correlated with spontaneous sensory and motor functional recovery (Dang et al., 2013; Harrison et al., 2013). In line with the ~60% reduction in infarct volume between 4 and 14 days, sensory and motor functional deficits in both wild type and NKO mice recovered by 14 days after SM stroke. Importantly, neuron-specific ablation of PTP1B hastened sensory and motor functional recovery, as little recovery was observed at 8 days in wild type mice, but near complete sensory and complete motor recovery were seen in the NKO mice at this time. Of note, at 4 days both wild type and NKO mice showed similar sensory and motor functional deficits. Thus, sensory and motor functional recovery occurred at least 4 days sooner in NKO mice compared to littermates. Further, our finding indicates that activation of the inflammatory responsive gene PTP1B in neurons impedes sensory and motor functional recovery.

While human and animal studies indicate that chronic inflammation is associated with anxiety and depression (Li et al., 2014b; Setiawan et al., 2015; McGuiness et al., 2016), we found that neuronal ablation of PTP1B is sufficient to prevent the appearance of anxiety and depression-like behaviors after limbic stroke. In addition, our observation of reduced PFC

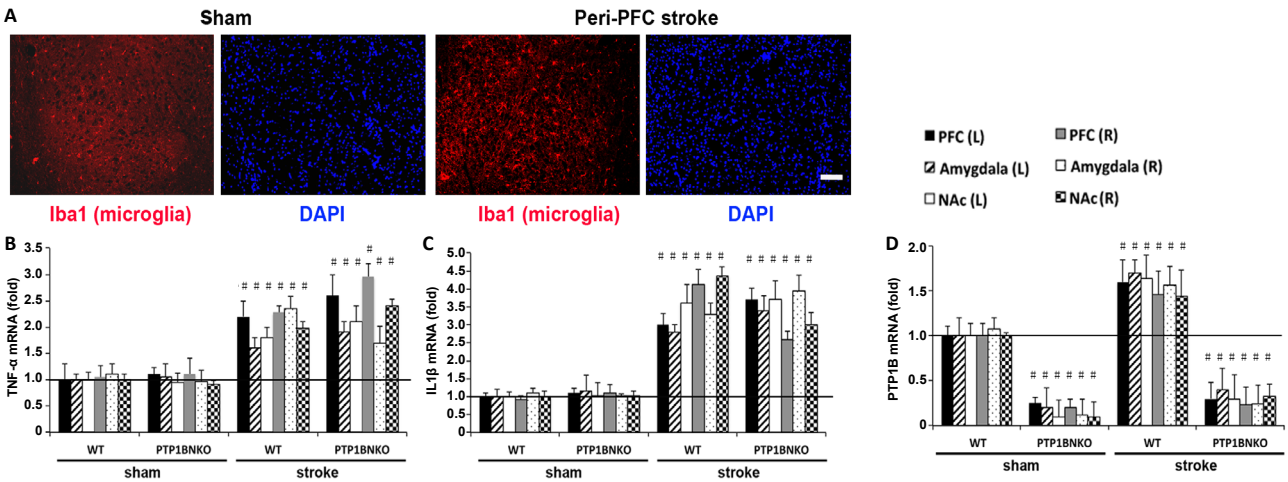


**Figure 4** | **Stroke activates a sustained global inflammatory response.**
(A) Immunofluorescence staining revealed activated microglia ($Iba1^+$) even on the contralateral side 6 weeks after peri-prefrontal cortex (PFC) stroke. Scale bar: 100 μm. qPCR reveals elevated mRNA for inflammatory cytokines (B, TNF-α; C, IL-1β) and for protein-tyrosine phosphatase 1B (PTP1B, D) in the PFC, amygdala and nucleus accumbens (NAc) after stroke. Tissues were sampled on the ipsilateral side (Left, L) and contralateral side (Right, R). Data were normalized to actin mRNA and expressed as a fold of WT-sham control. *n* = 5 mice/group. #$P < 0.05$, *vs.* WT sham for each subregion (Student's unpaired *t*-test).

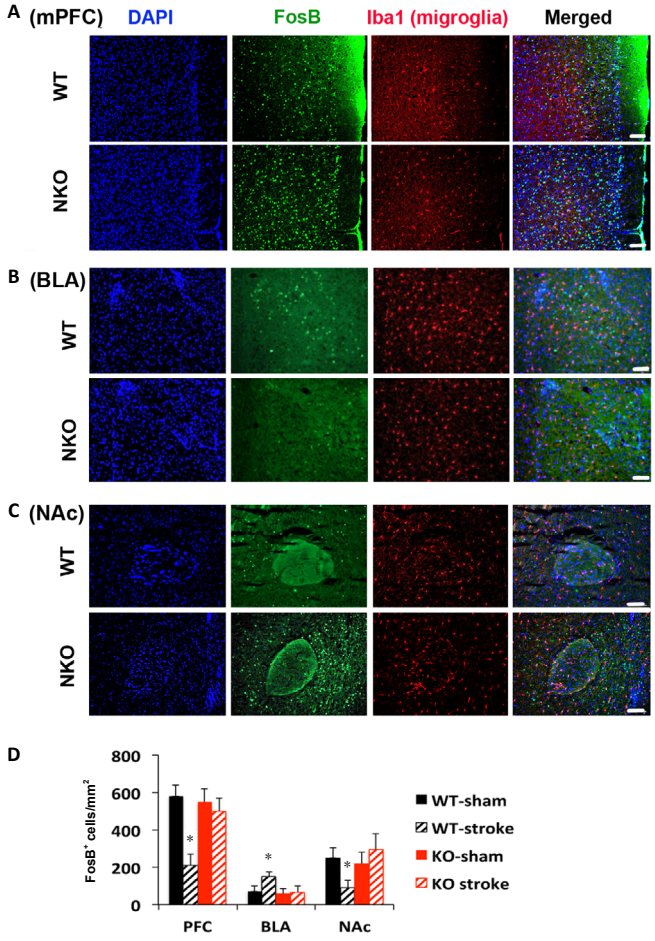


**Figure 5** | **Peri-PFC stroke causes widespread microglial activation with altered neuronal activity.**

Activated microglia (Iba1$^+$) were detected in PFC (A), amygdala (BLA, B) and nucleus accumbens (NAc, C) even 6 weeks after stroke. Neuronal activation revealed by the number of FosB$^+$ cells was quantified on the ipsilateral side. Merged panels show that FosB and Iba1 immunofluorescence is non-overlapping. Scale bars: 100 µm. (D) There were fewer FosB$^+$ neurons in the PFC and NAc of WT mice after stroke compared to PTP1BNKO mice. In contrast, there were more FosB$^+$ neurons in the BLA of WT compared to PTP1BNKO mice. $n$ = 4–5 mice/group. *$P$ < 0.05, *vs.* contralateral side (two-way analysis of variance followed by Tukey's *post hoc* test). BLA: Basolateral amygdala; PFC: prefrontal cortex; PTP1BNKO: protein-tyrosine phosphatase 1B knockout; KO: knockout; WT: wild type.

and NAc neuronal activity together with elevated BLA activity with post-stroke anxiety and depression aligns with reports that reduced activity in the PFC and NAc and elevated activity in the BLA are tied to anxiety and depression (Anand et al., 2005; Kim et al., 2011; Tan et al., 2011; Sripada et al., 2012; Admon et al., 2013; Goff et al., 2013; Shonesy et al., 2014; Vialou et al., 2014). Since these changes were not seen in NKO mice that do not develop post-stroke anxiety and depression-like behaviors, this indicates that PTP1B is required to alter neuronal activity linked to mood disorders after limbic stroke. An outstanding question that we have not addressed here is to what extent SM stroke contributes to anxiety- and depression-like behaviors.

By suppressing neuronal activity in the basolateral amygdala (BLA) and by promoting activity in the nucleus accumbens (NAc), respectively (Vialou et al., 2014), the prefrontal cortex (PFC) controls anxiety and depression (Bremner, 1999; Drevets, 2001; Keedwell et al., 2005; Mayberg et al., 2005; Feder et al., 2009). Functional magnetic resonance imaging showed that NAc activity declined following stress exposure and was associated with clinical symptoms of depression (Admon et al., 2013; Goff et al., 2013). Deep brain stimulation at the NAc has beneficial effects in treating resistant major depression (Nauczyciel et al., 2013). In a mouse model of anxiety and depression, stimulation of cortico-amygdala projections blocked an anxious phenotype, whereas stimulation of cortico-NAc projections produced an anti-depression-like effect (Vialou et al., 2014). Together, these studies highlight the importance of glutamatergic inputs from the PFC to the BLA and NAc to inhibit anxiety and depression, respectively. Positron emission tomography and single-photon emission computed tomography imaging studies showed that in most types of depression, including stroke-induced depression, the metabolism (a measure of neuronal activity) in the orbital-inferior prefrontal lobe is markedly reduced (Mayberg, 1994). Patients with left frontal cortical lesions have more severe mood disorders (Robinson et al., 1984). Human and animal studies show that decreased PFC pyramidal neuronal activity is associated with depression- and anxiety-like behaviors (Mayberg et al., 2005; Nahas et al., 2010), whereas increased PFC pyramidal neuron activity is associated with improved functional recovery after stroke (Clarke et al., 2014). These studies are consistent with our observations in wild type mice after left-sided peri-PFC stroke.

Many cellular mechanisms may be affected by neuronal PTP1B activation to account for the deleterious effects on sensory/motor recovery and the appearance of anxiety and depression after stroke. For example, stroke-induced PTP1B activity could undermine the beneficial effects of endogenous brain-derived neurotrophic factor (BDNF) signaling. BDNF is induced by stroke injury (Bejot et al., 2011) and BDNF/trkB signaling is an important self-protective mechanism that promotes neuronal survival, neuroplasticity (e.g., sensorimotor cortex reorganization (Murphy and Corbett, 2009)), and motor functional recovery after stroke (Schabitz et al., 2007; Ploughman et al., 2009; Clarkson et al., 2011). However, PTP1B dephosphorylates and inactivates the BDNF receptor trkB (Ozek et al., 2014) and this would delay functional recovery. In addition, PTP1B impairs the cellular response to insulin by dephosphorylating the insulin receptor and its substrate IRS1 (Moller, 2001; Bence et al., 2006; Pandey et al., 2013; Qin et al., 2015a; Kwon et al., 2018). Insulin signaling regulates trafficking of AMPA glutamate receptors that is important for synaptic plasticity (Ahmadian et al., 2004). In line with this notion, diabetes patients have poor functional recovery after stroke (Jorgensen et al., 1994). In addition, PTP1B affects many other receptors and signaling molecules that could affect stroke recovery including EphA3 (Nievergall et al., 2010), EphA5 (Liu et al., 2014), ErbB2 (Bentires-Alj and Neel, 2007), IGF-1 (Fan et al., 2013), Stat5 phosphorylation (Johnson et al., 2010), store-operated calcium entry (Koss et al., 2013), among many others. Lastly, elevated neuronal PTP1B would also hinder the mGluR5-dependent production of endocannabinoid (Qin et al., 2015b). Endocannabinoids are produced by postsynaptic neurons to inhibit presynaptic neurotransmitter release and thereby modulate neuronal activity. Endocannabinoid production is important in mood regulation and insufficient endocannabinoid signaling is associated with affective disorders (Qin et al., 2015b). Whether PTP1B activation alters neuronal excitability via endocannabinoid production or by other mechanisms are important questions to be addressed in future studies. It is worth noting that activation of PTP1B in parvalbumin inhibitory neurons affects repetitive and social behaviors (Zhang et al., 2020). To what extent PTP1B activation in parvalbumin and other inhibitory neurons affects recovery from stroke remains to be determined.

Taken together, our study shows that neuronal PTP1B directly influences stroke recovery and suggests that targeting PTP1B could improve both sensory and motor functional recovery as well as prevent mood disorders (anxiety and depression) associated with stroke injury. Although it is not tested here,

loss of cognitive function is a common stroke outcome. Our recent report demonstrates that elevated PTP1B function in neurons is associated with cognitive decline in the context of Alzheimer's disease with amyloid beta pathology in hAPP-J20 mice and importantly, systemic administration of Trodusquemine, a highly selective PTP1B inhibitor (Krishnan et al., 2014), rescued cognitive function in these mice (Ricke et al., 2020). Other compounds that inhibit PTP1B have been described, including Claramine (Qin et al., 2015a) and KY-226 (Sun et al., 2018). KY226 (Sun et al., 2018) was reported to reduce ischemic reperfusion damage, although whether this compound has any off-target effect remains to be determined. Trodusquemine has 200-fold selectivity for PTP1B compared to its closest homolog TC-PTP (Krishnan et al., 2014). Trodusquemine can readily cross the blood-brain barrier (Ahima et al., 2002) with a rapid (< 1 hour, after intraperitoneal injection) and long-acting (> 1 week) anxiolytic effect in mice with aberrantly elevated PTP1B (Qin et al., 2015b), but no measurable effect in healthy WT mice (Pandey et al., 2013, 2014; Qin et al., 2015a, b). Importantly, since Trodusquemine has undergone clinical trials for safety and for the treatment of obesity with no adverse outcomes (Nguyen et al., 2013), should future studies demonstrate a therapeutic effect of Trodusquemine in preclinical stroke models, it could be readily repurposed for stroke treatment.

## Conclusion

Our study has identified neuronal PTP1B as a key component that not only hinders sensory and motor functional recovery, but also accounts for the development of anxiety and depression after stroke. While the mechanisms affected by neuronal PTP1B activation remain to be elucidated, our study suggests that PTP1B may be a promising target to improve recovery, particularly in the first week after stroke.

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## References

Admon R, Lubin G, Rosenblatt JD, Stern O, Kahn I, Assaf M, Hendler T (2013) Imbalanced neural responsivity to risk and reward indicates stress vulnerability in humans. Cereb Cortex 23:28-35.
Ahima RS, Patel HR, Takahashi N, Qi Y, Hileman SM, Zasloff MA (2002) Appetite suppression and weight reduction by a centrally active aminosterol. Diabetes 51:2099-2104.
Ahmadian G, Ju W, Liu L, Wyszynski M, Lee SH, Dunah AW, Taghibiglou C, Wang Y, Lu J, Wong TP, Sheng M, Wang YT (2004) Tyrosine phosphorylation of GluR2 is required for insulin-stimulated AMPA receptor endocytosis and LTD. EMBO J 23:1040-1050.
Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L, Mathews VP, Kalnin A, Lowe MJ (2005) Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. Biol Psychiatry 57:1079-1088.
Bejot Y, Mossiat C, Giroud M, Prigent-Tessier A, Marie C (2011) Circulating and brain BDNF levels in stroke rats. Relevance to clinical studies. PLoS One 6:e29405.
Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, Kahn BB (2006) Neuronal PTP1B regulates body weight, adiposity and leptin action. Nat Med 12:917-924.
Bentires-Alj M, Neel BG (2007) Protein-tyrosine phosphatase 1B is required for HER2/Neu-induced breast cancer. Cancer Res 67:2420-2424.
Bremner JD (1999) Does stress damage the brain? Biol Psychiatry 45:797-805.
Casanova E, Fehsenfeld S, Mantamadiotis T, Lemberger T, Greiner E, Stewart AF, Schutz G (2001) A CamKIIalpha iCre BAC allows brain-specific gene inactivation. Genesis 31:37-42.
Castagne V, Moser P, Roux S, Porsolt RD (2011) Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Curr Protoc Neurosci Chapter 8:Unit 8 10A.
Chen HH, Xu J, Safarpour F, Stewart AF (2007a) LMO4 mRNA stability is regulated by extracellular ATP in F11 cells. Biochem Biophys Res Commun 357:56-61.
Chen HH, Schock SC, Xu J, Safarpour F, Thompson CS, Stewart AF (2007b) Extracellular ATP-dependent upregulation of the transcription cofactor LMO4 promotes neuron survival from hypoxia. Exp Cell Res 313:3106-3116.
Chen HH, Keyhanian K, Zhou X, Vilmundarson RO, Almontashiri NA, Cruz S, Pandey NR, Yap NL, Ho T, Stewart CA, Huang H, Hari A, Geoffrion M, McPherson R, Rayner KJ, Stewart AF (2015) IRF2BP2 reduces macrophage inflammation and susceptibility to atherosclerosis. Circ Res 117:671-683.
Chollet F, Tardy J, Albucher JF, Thalamas C, Berard E, Lamy C, Bejot Y, Deltour S, Jaillard A, Niclot P, Guillon B, Moulin T, Marque P, Pariente J, Arnaud C, Loubinoux I (2011) Fluoxetine for motor recovery after acute ischaemic stroke (FLAME): a randomised placebo-controlled trial. Lancet Neurol 10:123-130.
Clarke J, Langdon KD, Corbett D (2014) Early poststroke experience differentially alters periinfarct layer II and III cortex. J Cereb Blood Flow Metab 34:630-637.
Clarkson AN, Overman JJ, Zhong S, Mueller R, Lynch G, Carmichael ST (2011) AMPA receptor-induced local brain-derived neurotrophic factor signaling mediates motor recovery after stroke. J Neurosci 31:3766-3775.
Collaboration FT (2019) Effects of fluoxetine on functional outcomes after acute stroke (FOCUS): a pragmatic, double-blind, randomised, controlled trial. Lancet 393:265-274.
Cruz SA, Qin Z, Stewart AFR, Chen HH (2018) Dabrafenib, an inhibitor of RIP3 kinase-dependent necroptosis, reduces ischemic brain injury. Neural Regen Res 13:252-256.
Cruz SA, Hari A, Qin Z, Couture P, Huang H, Lagace DC, Stewart AFR, Chen HH (2017) Loss of IRF2BP2 in microglia increases inflammation and functional deficits after focal ischemic brain injury. Front Cell Neurosci 11:201.
Dang C, Liu G, Xing S, Xie C, Peng K, Li C, Li J, Zhang J, Chen L, Pei Z, Zeng J (2013) Longitudinal cortical volume changes correlate with motor recovery in patients after acute local subcortical infarction. Stroke 44:2795-2801.
Das RR, Seshadri S, Beiser AS, Kelly-Hayes M, Au R, Himali JJ, Kase CS, Benjamin EJ, Polak JF, O'Donnell CJ, Yoshita M, D'Agostino RB, Sr., DeCarli C, Wolf PA (2008) Prevalence and correlates of silent cerebral infarcts in the Framingham offspring study. Stroke 39:2929-2935.
Drevets WC (2001) Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. Curr Opin Neurobiol 11:240-249.
Fan G, Lin G, Lucito R, Tonks NK (2013) Protein-tyrosine phosphatase 1B antagonized signaling by insulin-like growth factor-1 receptor and kinase BRK/PTK6 in ovarian cancer cells. J Biol Chem 288:24923-24934.
Feder A, Nestler EJ, Charney DS (2009) Psychobiology and molecular genetics of resilience. Nat Rev Neurosci 10:446-457.
Franklin K, Paxinos G (2008) The mouse brain in stereotaxic coordinates. 3rd ed. San Diego, USA: Academic Press.
Gerhard A, Schwarz J, Myers R, Wise R, Banati RB (2005) Evolution of microglial activation in patients after ischemic stroke: a [11C](R)-PK11195 PET study. Neuroimage 24:591-595.
Goff B, Gee DG, Telzer EH, Humphreys KL, Gabard-Durnam L, Flannery J, Tottenham N (2013) Reduced nucleus accumbens reactivity and adolescent depression following early-life stress. Neuroscience 249:129-138.
Gomez-Smith M, Qin Z, Zhou X, Schock SC, Chen HH (2010) LIM domain only 4 protein promotes granulocyte colony-stimulating factor-induced signaling in neurons. Cell Mol Life Sci 67:949-957.
Greifzu F, Schmidt S, Schmidt KF, Kreikemeier K, Witte OW, Lowel S (2011) Global impairment and therapeutic restoration of visual plasticity mechanisms after a localized cortical stroke. Proc Natl Acad Sci U S A 108:15450-15455.
Gulyas B, Toth M, Schain M, Airaksinen A, Vas A, Kostulas K, Lindstrom P, Hillert J, Halldin C (2012) Evolution of microglial activation in ischaemic core and peri-infarct regions after stroke: a PET study with the TSPO molecular imaging biomarker [((11))C] vinpocetine. J Neurol Sci 320:110-117.
Harrison TC, Silasi G, Boyd JD, Murphy TH (2013) Displacement of sensory maps and disorganization of motor cortex after targeted stroke in mice. Stroke 44:2300-2306.
Hodes GE, Kana V, Menard C, Merad M, Russo SJ (2015) Neuroimmune mechanisms of depression. Nat Neurosci 18:1386-1393.

Johnson KJ, Peck AR, Liu C, Tran TH, Utama FE, Sjolund AB, Schaber JD, Witkiewicz AK, Rui H (2010) PTP1B suppresses prolactin activation of Stat5 in breast cancer cells. Am J Pathol 177:2971-2983.
Jorgensen H, Nakayama H, Raaschou HO, Olsen TS (1994) Stroke in patients with diabetes. The Copenhagen Stroke Study. Stroke 25:1977-1984.
Juang HT, Chen PC, Chien KL (2015) Using antidepressants and the risk of stroke recurrence: report from a national representative cohort study. BMC Neurol 15:86.
Keedwell PA, Andrew C, Williams SC, Brammer MJ, Phillips ML (2005) The neural correlates of anhedonia in major depressive disorder. Biol Psychiatry 58:843-853.
Kim MJ, Gee DG, Loucks RA, Davis FC, Whalen PJ (2011) Anxiety dissociates dorsal and ventral medial prefrontal cortex functional connectivity with the amygdala at rest. Cereb Cortex 21:1667-1673.
Kiphuth IC, Utz KS, Noble AJ, Kohrmann M, Schenk T (2014) Increased prevalence of posttraumatic stress disorder in patients after transient ischemic attack. Stroke 45:3360-3366.
Koss DJ, Riedel G, Bence K, Platt B (2013) Store-operated Ca(2+) entry in hippocampal neurons: Regulation by protein tyrosine phosphatase PTP1B. Cell Calcium 53:125-138.
Krishnan N, Koveal D, Miller DH, Xue B, Akshinthala SD, Kragelj J, Jensen MR, Gauss CM, Page R, Blackledge M, Muthuswamy SK, Peti W, Tonks NK (2014) Targeting the disordered C terminus of PTP1B with an allosteric inhibitor. Nat Chem Biol 10:558-566.
Krishnan V, Nestler EJ (2008) The molecular neurobiology of depression. Nature 455:894-902.
Kronenberg G, Balkaya M, Prinz V, Gertz K, Ji S, Kirste I, Heuser I, Kampmann B, Hellmann-Regen J, Gass P, Sohr R, Hellweg R, Waeber C, Juckel G, Hortnagl H, Stumm R, Endres M (2012) Exofocal dopaminergic degeneration as antidepressant target in mouse model of poststroke depression. Biol Psychiatry 72:273-281.
Kwon H, Jang D, Choi M, Lee J, Jeong K, Pak Y (2018) Alternative translation initiation of Caveolin-2 desensitizes insulin signaling through dephosphorylation of insulin receptor by PTP1B and causes insulin resistance. Biochim Biophys Acta 1864:2169-2182.
Li H, Zhang N, Lin HY, Yu Y, Cai QY, Ma L, Ding S (2014a) Histological, cellular and behavioral assessments of stroke outcomes after photothrombosis-induced ischemia in adult mice. BMC Neurosci 15:58.
Li Z, Ma L, Kulesskaya N, Voikar V, Tian L (2014b) Microglia are polarized to M1 type in high-anxiety inbred mice in response to lipopolysaccharide challenge. Brain Behav Immun 38:237-248.
Liu S, Xi Y, Bettaieb A, Matsuo K, Matsuo I, Kulkarni RN, Haj FG (2014) Disruption of protein-tyrosine phosphatase 1B expression in the pancreas affects beta-cell function. Endocrinology 155:3329-3338.
Mayberg HS (1994) Frontal lobe dysfunction in secondary depression. J Neuropsychiatry Clin Neurosci 6:428-442.
Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, Schwalb JM, Kennedy SH (2005) Deep brain stimulation for treatment-resistant depression. Neuron 45:651-660.
McGuiness B, Gibney SM, Beumer W, Versnel MA, Sillaber I, Harkin A, Drexhage HA (2016) Exaggerated increases in microglia proliferation, brain inflammatory response and sickness behaviour upon lipopolysaccharide stimulation in non-obese diabetic mice. Neuroimmunomodulation 23:137-150.
Mena H, Cadavid D, Rushing EJ (2004) Human cerebral infarct: a proposed histopathologic classification based on 137 cases. Acta Neuropathol 108:524-530.
Mikami K, Jorge RE, Adams HP, Jr., Davis PH, Leira EC, Jang M, Robinson RG (2011) Effect of antidepressants on the course of disability following stroke. Am J Geriatr Psychiatry 19:1007-1015.
Moller DE (2001) New drug targets for type 2 diabetes and the metabolic syndrome. Nature 414:821-827.
Mortensen JK, Larsson H, Johnsen SP, Andersen G (2013) Post stroke use of selective serotonin reuptake inhibitors and clinical outcome among patients with ischemic stroke: a nationwide propensity score-matched follow-up study. Stroke 44:420-426.
Murphy TH, Corbett D (2009) Plasticity during stroke recovery: from synapse to behaviour. Nat Rev Neurosci 10:861-872.
Nahas Z, Anderson BS, Borckardt J, Arana AB, George MS, Reeves ST, Takacs I (2010) Bilateral epidural prefrontal cortical stimulation for treatment-resistant depression. Biol Psychiatry 67:101-109.
Nauczyciel C, Robic S, Dondaine T, Verin M, Robert G, Drapier D, Naudet F, Millet B (2013) The nucleus accumbens: a target for deep brain stimulation in resistant major depressive disorder. J Mol Psychiatry 1:17.
Nievergall E, Janes PW, Stegmayer C, Vail ME, Haj FG, Teng SW, Neel BG, Bastiaens PI, Lackmann M (2010) PTP1B regulates Eph receptor function and trafficking. J Cell Biol 191:1189-1203.
Ozek C, Kanoski SE, Zhang ZY, Grill HJ, Bence KK (2014) Protein-tyrosine phosphatase 1B (PTP1B) is a novel regulator of central brain-derived neurotrophic factor and tropomyosin receptor kinase B (TrkB) signaling. J Biol Chem 289:31682-31692.
Pandey NR, Zhou X, Qin Z, Zaman T, Gomez-Smith M, Keyhanian K, Anisman H, Brunel JM, Stewart AF, Chen HH (2013) The LIM domain only 4 protein is a metabolic responsive inhibitor of protein tyrosine phosphatase 1B that controls hypothalamic leptin signaling. J Neurosci 33:12647-12655.
Pandey NR, Zhou X, Zaman T, Cruz SA, Qin Z, Lu M, Keyhanian K, Brunel JM, Stewart AF, Chen HH (2014) LMO4 is required to maintain hypothalamic insulin signaling. Biochem Biophys Res Commun 450:666-672.
Ploughman M, Windle V, MacLellan CL, White N, Dore JJ, Corbett D (2009) Brain-derived neurotrophic factor contributes to recovery of skilled reaching after focal ischemia in rats. Stroke 40:1490-1495.
Qin Z, Pandey NR, Zhou X, Stewart CA, Hari A, Huang H, Stewart AF, Brunel JM, Chen HH (2015a) Functional properties of Claramine: A novel PTP1B inhibitor and insulin-mimetic compound. Biochem Biophys Res Commun 458:21-27.
Qin Z, Zhou X, Pandey NR, Vecchiarelli HA, Stewart CA, Zhang X, Lagace DC, Brunel JM, Beique JC, Stewart AF, Hill MN, Chen HH (2015b) Chronic stress induces anxiety via an amygdalar intracellular cascade that impairs endocannabinoid signaling. Neuron 85:1319-1331.
Renthal W, Carle TL, Maze I, Covington HE, 3rd, Truong HT, Alibhai I, Kumar A, Montgomery RL, Olson EN, Nestler EJ (2008) Delta FosB mediates epigenetic desensitization of the c-fos gene after chronic amphetamine exposure. J Neurosci 28:7344-7349.
Ricke KM, Cruz SA, Qin Z, Farrokhi K, Sharmin F, Zhang L, Zasloff MA, Stewart AFR, Chen HH (2020) Neuronal Protein Tyrosine Phosphatase 1B hastens Amyloid beta-associated Alzheimer's disease in mice. J Neurosci 40:1581-1593.
Robinson RG, Kubos KL, Starr LB, Rao K, Price TR (1984) Mood disorders in stroke patients. Importance of location of lesion. Brain 107 (Pt 1):81-93.
Schabitz WR, Steigleder T, Cooper-Kuhn CM, Schwab S, Sommer C, Schneider A, Kuhn HG (2007) Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis. Stroke 38:2165-2172.
Schock SC, Xu J, Duquette PM, Qin Z, Lewandowski AJ, Rai PS, Thompson CS, Seifert EL, Harper ME, Chen HH (2008) Rescue of neurons from ischemic injury by peroxisome proliferator-activated receptor-gamma requires a novel essential cofactor LMO4. J Neurosci 28:12433-12444.
Setiawan E, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Rajkowska G, Suridjan I, Kennedy JL, Rekkas PV, Houle S, Meyer JH (2015) Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. JAMA Psychiatry 72:268-275.
Shonesy BC, Bluett RJ, Ramikie TS, Baldi R, Hermanson DJ, Kingsley PJ, Marnett LJ, Winder DG, Colbran RJ, Patel S (2014) Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. Cell Rep 9:1644-1653.
Song GJ, Jung M, Kim JH, Park H, Rahman MH, Zhang S, Zhang ZY, Park DH, Kook H, Lee IK, Suk K (2016) A novel role for protein tyrosine phosphatase 1B as a positive regulator of neuroinflammation. J Neuroinflammation 13:86.
Sripada RK, King AP, Garfinkel SN, Wang X, Sripada CS, Welsh RC, Liberzon I (2012) Altered resting-state amygdala functional connectivity in men with posttraumatic stress disorder. J Psychiatry Neurosci 37:241-249.
Sun M, Izumi H, Shinoda Y, Fukunaga K (2018) Neuroprotective effects of protein tyrosine phosphatase 1B inhibitor on cerebral ischemia/reperfusion in mice. Brain Res 1694:1-12.
Tan H, Lauzon NM, Bishop SF, Chi N, Bechard M, Laviolette SR (2011) Cannabinoid transmission in the basolateral amygdala modulates fear memory formation via functional inputs to the prelimbic cortex. J Neurosci 31:5300-5312.
Terroni L, Amaro E, Iosifescu DV, Tinone G, Sato JR, Leite CC, Sobreiro MF, Lucia MC, Scaff M, Fraguas R (2011) Stroke lesion in cortical neural circuits and post-stroke incidence of major depressive episode: a 4-month prospective study. World J Biol Psychiatry 12:539-548.
Tobinick E (2011) Rapid improvement of chronic stroke deficits after perispinal etanercept: three consecutive cases. CNS Drugs 25:145-155.
Tsunekawa T, Banno R, Mizoguchi A, Sugiyama M, Tominaga T, Onoue T, Hagiwara D, Ito Y, Iwama S, Goto M, Suga H, Sugimura Y, Arima H (2017) Deficiency of PTP1B attenuates hypothalamic inflammation via activation of the JAK2-STAT3 pathway in microglia. EBioMedicine 16:172-183.
Vahid-Ansari F, Lagace DC, Albert PR (2016) Persistent post-stroke depression in mice following unilateral medial prefrontal cortical stroke. Transl Psychiatry 6:e863.
Vialou V, Bagot RC, Cahill ME, Ferguson D, Robison AJ, Dietz DM, Fallon B, Mazei-Robison M, Ku SM, Harrigan E, Winstanley CA, Joshi T, Feng J, Berton O, Nestler EJ (2014) Prefrontal cortical circuit for depression- and anxiety-related behaviors mediated by cholecystokinin: role of DeltaFosB. J Neurosci 34:3878-3887.
Vialou V et al. (2010) DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. Nat Neurosci 13:745-752.
Watson BD, Dietrich WD, Busto R, Wachtel MS, Ginsberg MD (1985) Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol 17:497-504.
Wu RH, Feng C, Xu Y, Hua T, Liu XY, Fang M (2014) Late-onset depression in the absence of stroke: associated with silent brain infarctions, microbleeds and lesion locations. Int J Med Sci 11:587-592.
Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB (2008) Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J Biol Chem 283:14230-14241.
Zaman T, Zhou X, Pandey NR, Qin Z, Keyhanian K, Wen K, Courtney RD, Stewart AF, Chen HH (2014) LMO4 is essential for paraventricular hypothalamic neuronal activity and calcium channel expression to prevent hyperphagia. J Neurosci 34:140-148.
Zhang L, Qin Z, Ricke KM, Cruz SA, Stewart AFR, Chen HH (2020) Hyperactivated PTP1B phosphatase in parvalbumin neurons alters anterior cingulate inhibitory circuits and induces autism-like behaviors. Nat Commun 11:1017.
Zhou X, Gomez-Smith M, Qin Z, Duquette PM, Cardenas-Blanco A, Rai PS, Harper ME, Tsai EC, Anisman H, Chen HH (2012) Ablation of LMO4 in glutamatergic neurons impairs leptin control of fat metabolism. Cell Mol Life Sci 69:819-828.
Zhu X, Zhou Y, Tao R, Zhao J, Chen J, Liu C, Xu Z, Bao G, Zhang J, Chen M, Shen J, Cheng C, Zhang D (2015) Upregulation of PTP1B after rat spinal cord injury. Inflammation 38:1891-1902.

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