Full Length Article

# Skin-brain axis signaling mediates behavioral changes after skin wounding

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ABSTRACT

Patients with chronic wounds often have associated cognitive dysfunction and depression with an as yet unknown mechanism for this association. To address the possible causality of skin wounding inducing these changes, behavior and cognitive functions of female C57BL/6 mice with an excisional skin wound were compared to unwounded animals. At six days post wounding, animals exhibited anxiety-like behaviors, impaired recognition memory, and impaired coping behavior. Wounded animals also had concomitant increased hippocampal expression of Tnfa, the pattern recognition receptor (PRR) Nod2, the glucocorticoid receptors GR/Nr3c1 and Nr3c2. Prefrontal cortex serotonin and dopamine turnover were increased on day six post-wounding. In contrast to the central nervous system (CNS) findings, day six post -wounding serum catecholamines did not differ between wounded and unwounded animals, nor did levels of the stress hormone corticosterone, TNFα, or TGFβ. Serum IL6 levels were, however elevated in the wounded animals. These findings provide evidence of skin-to-brain signaling, mediated either by elevated serum IL6 or a direct neuronal signaling from the periphery to the CNS, independent of systemic mediators. Wounding in the periphery is associated with an altered expression of inflammatory mediators and PRR genes in the hippocampus, which may be responsible for the observed behavioral deficits.

## 1. Introduction

The 'skin-brain axis' communication network has been recognized as a mechanism by which the central nervous system (CNS) response to psychological stress results in an afferent relay and physiological outcome in the skin (Paus et al., 2006; Pavlovic et al., 2008; Martins et al., 2020; Rodriguez-Vallecillo and Woodbury-Farina, 2014; Chapman and Moynihan, 2009). Stress, through a CNS-mediated mechanism, has been shown to exacerbate peripheral cutaneous manifestations of atopic dermatitis, psoriasis, and even hair growth (Martins et al., 2020; Alexopoulos and Chrousos, 2016; Ayasse et al., 2020; Paus, 2016; Pondeljak and Lugović-Mihić, 2020; Theoharides, 2020; Torales et al., 2020; Yang and Zheng, 2020; Zeiser et al., 2021). However, in addition to the skin being a target for stress responses, bi-directional signaling is described

wherein the skin itself, through the localized production of neuro-immunomodulators and stress catecholamines, can initiate stress signaling to the brain (Paus et al., 2006; Chen and Lyga, 2014). The complete implication of this skin-to- brain signaling has not been fully explored.

Here we consider the skin-brain axis as a possible novel paradigm that could reveal the mechanism linking chronic skin wounds to the noted associated cognitive dysfunction. Depression, anxiety and cognitive dysfunction have been associated with chronic skin ulcers of venous or diabetic etiologies (Green et al., 2014; Kouris et al., 2016; Maydick and Acee, 2016; Moffatt et al., 2009; Natovich et al., 2016; Platsidaki et al., 2017; Renner and Erfurt-Berge, 2017; Souza Nogueira et al., 2009; Udovichenko et al., 2017; Zhou and Jia, 2016) (Williams et al., 2010). Neither the mechanism for this association, nor the directional pattern of

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causality is known. The leading neurobiological mechanism to explain primary mood disorders is the monoamine hypothesis which posits that depression and anxiety are associated with alterations in CNS serotonin, epinephrine and dopamine signaling (Jans et al., 2007; Hillhouse and Porter, 2015). Monoamines, especially dopamine, are essential for memory formation (Wise, 2004) and altered CNS dopamine signaling has been associated with cognitive dysfunction in a number of diseases (Calabresi et al., 2006; Braver and Cohen, 1999; Pan et al., 2019). Additionally, systemic levels of inflammatory cytokines have also been associated with cognitive deficits in patients with post-traumatic stress disorder, and major depressive disease (Hadian et al., 2020; Imai et al., 2018; Roohi et al., 2021). We hypothesized that the affective and cognitive symptoms in subjects with chronic skin wounds associate with alterations in CNS monamines, or with systemic elevation of inflammatory cytokines, and sought to determine the signaling pathways between peripheral wounds and the central nervous system. Therefore, we set out to determine the effect of skin wounding on behavior and identify signaling mechanisms to determine potential causality for the associated findings. Since a large percentage (>40%) of patients with chronic wounds exhibit cognitive disorders that adversely affect their clinical outcome (35) (Gonzalez et al., 2008; Lin et al., 2004; Pearson et al., 2014), finding a link between the skin wound and behavioral abnormalities could explain the observed findings and lead to novel approaches for interrupting this signaling axis.

## 2. Methods

### 2.1. Animals

Female C57BL/6 (14–15 week old, 20–24 g) mice were obtained from Jackson Laboratory. Animals were allowed to acclimate for 1 week after transfer from the vendor to the UC Davis vivarium. Mice were housed in cages lined with chip bedding on a 12 h light/dark cycle (lights on at 08:00) with free access to food and water. Animals were kept in the containment unit of the animal facility and all experiments were performed in a biosafety cabinet. All procedures and protocols were reviewed and approved by the institution's IACUC.

### 2.2. Study design

Mice were randomly assigned to wounded or unwounded groups to avoid bias in study design. Sample size calculation was performed using G*Power (3.1) based on data collected in our previous behavioral experiments showing impairments, where $\alpha = 0.05$, power $[1-\beta] = 0.8$ (Keogh et al., 2021a, 2021b). Two days prior to wounding surgery, all mice were sedated with 3–5% isoflourane and the dorsal surface was shaved, depilated wounded, and bandaged. Behavioral testing was performed 3 and 6 days post wounding. Control animals were treated identically but not wounded. Despite identical bandaging on control and wounded animals, the wounds on the dorsum of the animals were visible preventing blinding of the behavioral experimenter, who were, however, blind to the experimental outcome during analysis. A subset of videos was analyzed by a second experimenter, unfamiliar with the experimental paradigm, to confirm findings. Analysis of serum, hippocampal and cortical analytes were performed by experimenters blinded to the study design.

### 2.3. Wounding

Two days prior to wounding surgery, all mice were sedated with 3–5% isoflourane and the dorsal surface was shaved. Depilatory cream was applied and removed within 5–10 s of application to remove remaining hair. For surgery, all mice were anesthetized with isoflourane. Buprenorphine (0.05 mg/kg) was administered subcutaneously prior to wounding procedure. Mice were then sterilely prepped with iodine and ethanol wipes. An 8 mm sterile skin biopsy punch instrument was used to create two bilateral full thickness wounds on the dorsal skin. Skin Shield® (Insight Pharmaceuticals Corp) was applied to the outside edges of each wound to ensure adherence of TegaDerm™ (3 M Medical) to the skin. TegaDerm™ wound dressing was then applied to cover both wounds. Unwounded mice were prepped for surgery and given an identical dose of buprenorphine, but only had Skin Shield ® and Tega-Derm ™ applied to their backs without receiving any wound. At the end of the experiment, mice were anesthetized with an overdose of iso-flourane. Blood was then drawn via retro-orbital bleeding until totally exsanguinated. Death was then confirmed by cervical dislocation.

### 2.4. Behavioral testing

Each mouse was subjected to all three tests, with a 1-h rest period between tests. After the last test, mice were allowed to rest for ~1.5 h before sacrifice and tissue collection. The order of tests was the same for each animal, and progressed from least stressful to most; Light/Dark (L/D) Box, then Novel Object Recognition (NOR) Task, then Forced Swim Test (FST). All behavior tests were administered between 7:00am and 1:00pm, and tissue collections happened between 2:30 and 5:00pm. Euthanasia and tissue collection were performed on the day of testing to ensure that the behavior data and biological data were temporally correlated.

### 2.5. Forced swim test (FST)

The FST was used to assess coping behavior following an inescapable stressor. While not a direct surrogate for depressive-like behavior, impaired FST responses are known to be improved following treatment with anti-depressants (de Kloet and Molendijk, 2016). When placed in a container filled with water, a rodent will make efforts to escape, but eventually will give up and become immobile, which is considered to model behavioral despair. (Yankelevitch-Yahav et al., 2015; Gareau et al., 2011; Emge et al., 2016; Smith et al., 2014). Mice were placed into a 1 L glass beaker with room temperature (20–22C) water and allowed to swim for 6 min with video camera recording of the mouse's activity. The first 2 min of activity were not scored, to exclude the initial adaptation period of the test and the last 4 min of the testing period analyzed manually. During the behavioral analysis, mobility time (any movements other than those necessary to balance the body and keep the head above the water) was measured (Pusceddu et al., 2019).

Female C57BL/6 (14–15 week old, 20–24 g) mice were randomly assigned to experimental groups samples/animals were assigned randomly to various experimental groups (and the specific method of randomization); 2) the data collected was processed randomly and appropriately blocked; 3) experimenters were blind to group assignment and outcome assignment; and 4) an appropriate sample size was computed when the study was being designed.

### 2.6. Novel object recognition (NOR)Task

Recognition memory was assessed using the highly validated NOR task (Ennaceur and Delacour, 1988). This test is based on the tendency of mice to investigate a novel object in the arena, rather than a known one (Gareau et al., 2011; Ahn et al., 2008). Exploratory behavior was video recorded (5 min) and analyzed for frequency of smell for each object. Objects used included: two metal drawer handles indistinguishable from each other (objects #1 and #2), and a different door handle of similar size, but different in shape and color (object #3). To avoid object bias, preliminary studies tested objects for equal preference. Behavioral assessment consisted of distinct training and testing phases.

### 2.7. Training (familiarization) phase

Mice were placed individually in a new, clean cage for habituation. After 5 min, objects #1 and #2 were placed in opposite corners of the

cage. Behavior was video recorded for 5 min, after which the objects were removed. Mice were rested for 45 min before the (next) testing phase.

### 2.8. Testing phase

After resting, mice were re-exposed to object #2, which was re-introduced into the cage (referred to now as object #2 B) along with a completely new object (object #3). Behavior was video recorded for 5 min. Memory was assessed as the frequency to explore object #2 B during the testing phase, compared with the new object #3. An exploration ratio was calculated (exploration ratio = freq. smell #3/(freq. smell #3 + freq. smell #2 B) × 100). This ratio represents the proportion of smelling bouts associated with the new object versus the old object. A ratio of 50% represents no discrimination between the two objects and impaired preference. Exploration of objects was defined as orientation towards the object with the nose of the mouse pointed towards the object within 2 cm. The training phase showed no preference between either object #1 or #2.

### 2.9. Light/dark (L/D) box

Anxiety-like behavior was assessed using the L/D box test (Bourin and Hascoet, 2003). Anxiety-like behavior is assessed as an increased tendency of the animal to remain in the dark box, rather than follow its natural tendency to explore a novel area that is brightly lit (Takao and Miyakawa, 2006). Mice were placed in a clean rat cage lined with bedding containing a light (2/3) and a dark (1/3) compartment. The mouse was introduced to the light compartment and the total time spent in each compartment was recorded by video camera for 10 min. This test lasts a total of 10 min/mouse and is performed once per mouse.

### 2.10. RNA extraction and purification

Immediately following exsanguination via retro orbital bleed, mice were rapidly decapitated and the whole brain removed for dissection. The cerebellum, hippocampus, and cortex were isolated and snap frozen in liquid nitrogen immediately. 10 mm biopsies including the wound were excised from the dorsal wound and snap frozen in liquid nitrogen immediately after being harvested from the animal. Using RNA Mini Kit (Qiagen, Germantown, MD) tissues were then placed in lysis buffer RLT (Qiagen) with lysis beads, then pulverized using the Bullet Blender (NextAdvance, Troy, NY). RNA was then extracted from the lysate according to the manufacturer's protocol. The resulting total RNA concentration and quality was then assessed via NanoDrop (Thermo Fisher).

### 2.11. cDNA synthesis and qPCR

100 ng of total RNA was reverse transcribed to cDNA using the High Capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). RNA was then diluted to 100ng/ul and 1 μl was used in each qPCR reaction. Additionally, 5 μl of SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA), 500 nM forward and reverse primers, and nuclease free water was added to 10 μl per reaction. Changes in gene expression were measured by qPCR using the CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Samples were heated to 95 °C for 30 s followed by 40 cycles of 95 °C for 15 s, and 60 °C for 30 s. Genetic expression analysis was performed using the ΔΔCt method and all samples were normalized to the 18s, and *Gapdh* genes. Reference gene stability was determined to be ideal, and all expression analysis was performed using the CFX Maestro analysis software (Bio-Rad, Hercules, CA). Primers are listed in Supplemental Methods.

### 2.12. Catecholamine determination

Neurochemicals in mouse cortex and serum were measured using ion-

pairing UHPLC with amperometric detection. For all sample types the catecholamines and serotonin, precursors, and metabolites were separated on an Acquity UPLC BEH C18 column, and different mobile phases were prepared for each of the three analyses. Briefly, cortex samples were homogenized in aqueous perchloric acid and proteins were removed by ultrafiltration prior to analysis; serum catecholamines were extracted via phenylboronic acid-silica solid-phase extraction (SPE) and eluted using 1% (m/v) acetic acid prior to analysis; serum tryptophan, serotonin, tryptamine, and 5-HIAA were analyzed after protein precipitation using acidified methanol followed by dilution in 0.1 N HClO4 containing 2 mM EDTA. For cortex neurochemicals and serum catecholamines we performed external standardization and 3,4-dihydroxbenzylamine was added to serum samples as a surrogate standard to account for losses during SPE. For serum tryptamines we performed internal standardization using N-methylserotonin to account for volume and transfer errors as well as variable injection volumes when operating in partial-loop mode. Quantitation was performed using peak areas, or peak area ratios in the case of serum tryptamines, and neurochemical amounts were normalized to tissue mass or serum volume and reported as pmol/gram tissue in cortex or nM for serum measurements. More detailed information is provided in the supplement.

### 2.13. Serum cytokine detection

Mice were anesthetized with 3% isoflurane and whole blood was extracted via retro orbital draw. The blood was then allowed to clot at room temperature for 15–20 min. The blood was then centrifuged at 1500 g for 10 min to separate serum. A cytometric bead assay was used to determine the serum concentration of TNFα (558299), TGFβ (560429), and IL6 (558301) (BD Biosciences). Assays were performed according to the manufacturers protocol without any changes.

### 2.14. Serum corticosterone analysis

Blood samples were collected as above for cytokine detection, and serum stored at −80 °C until analysis. Corticosterone levels were assayed using a commercially available enzyme immunoassay (EIA) kit (Corticosterone EIA Kit, ADI-900-097; Enzo Life Sciences, Farmingdale, NY, USA) in accordance with the manufacturer's instructions. Absorbance was read with a multimode plate reader (Synergy H1; BioTek Inc., Winooski, VT, USA) at 405 nm. Results are presented as pg/ml of serum.

### 2.15. Statistical analysis

Behavior testing data was analyzed with GraphPad Prism 9.0.1 software using the unpaired Mann-Whitney *U* test. qPCR data was analyzed with Bio-Rad CFX Maestro software and significance set at p < 0.05 using the unpaired T test. For statistical analysis of neurochemicals results, we used Student's t-test for heteroscedastic data. Differences were determined to be significant if p-values were less than 0.05. Pearson's correlation coefficient was used to determine significance for linear correlations. Linear correlations were determined to be significant if the test statistic was larger than the critical value corresponding to p = 0.05 taken from a Student T table.

### 2.16. Data handling

*N numbers*: N for each group is listed in each figure legend. *Outliers:* Outliers were identified using the ROUT (Q = 1%) method in Prism and there were 3 outliers removed from the serum CBA.

### 2.17. Study approval

All procedures and protocols were reviewed and approved by the institution's IACUC.

## 3. Results

### 3.1. Wounded animals develop cognitive and behavioral alterations

At three days post-wounding the only behavioral impairment identified was impaired recognition memory, identified in the NOR task, as determined by reduced exploration ratio of a novel versus a known object by the wounded animals, compared with sham-treated controls (Fig. 1 A, B, C). By six days post-wounding, however, in addition to decreased recognition memory (Fig. 1D), the wounded animals presented with anxiety-like behavior, as evidenced by decreased total time spent in the light compartment in the L/D box test compared with sham-treated controls (Fig. 1 F). A significant impairment in coping following an inescapable stressor, as evidenced by increased time spent immobile of the wounded animals in the FST was also observed (Fig. 1 E).

### 3.2. Hippocampal expression of inflammatory mediators is increased in wounded animals

Three days post-wounding there were no significant increases in inflammatory mediators in the hippocampi of wounded animals (Fig. 2 A). However, by day 6 post-wounding, animals demonstrated significant increases in hippocampal expression of the pro-inflammatory cytokine Tnfa the pattern recognition receptor (PRR) Nod2, the glucocorticoid receptors GR/Nr3c1 and Nr3c2. Expression of tryptophan hydroxylase, the rate limiting enzyme for 5HT synthesis, and Tgfb were also increased (Fig. 2 B).

### 3.3. IL6 is elevated in serum of wounded animals

To evaluate whether wounding induces an increase in the levels of systemic inflammatory mediators, serum was collected on Days 3 and 6 post-wounding from both wounded and control animals. There were no differences in any of the cytokines measured or corticosterone levels between the wounded and unwounded animals on Day 3 (Supplemental Figure 2). On Day 6, however, IL-6 was elevated in wounded animals

relative to the unwounded controls. Interestingly, however, the increase in expression of TGFβ and TNFα in the hippocampus was not mirrored in the serum. The stress hormone, corticosterone was also not elevated in the serum of the wounded animals (Fig. 3).

### 3.4. Serotonin and dopamine metabolites are upregulated in cerebral cortex of wounded mice, but not in serum

Catecholamines were measured in the cerebral cortices of wounded and sham-wounded animals at days 3 or 6 post-wounding. There was no difference in the levels of neuroamines in either the cortices or serum of wounded vs. unwound animals on day 3 (Supplemental Fig. 1A and B). However, serotonin pathway metabolites were increased in the day 6 wounded animals' cortices relative to unwounded controls as evidenced by increase in tryptophan precursor and of the serotonin metabolite 5-HIAA (Fig. 4A). The dopamine metabolite, HVA was also elevated in the cortex of wounded animals on day 6 post-wounding (Fig. 4 A). Serum neuroamine metabolites were not significantly different in wounded vs unwounded animals on day 6 post-wounding (Fig. 4B). In wounded animals, the lower levels of 5-HIAA and higher levels of serotonin in the cerebral cortex (Fig. 4C) trended with better performance (more time in the light compartment), while higher levels of 5-HIAA trended with poorer performance on the FST (more time immobile).

## 4. Discussion

This study demonstrates that skin wounding in mice is associated with the development of memory deficits, anxiety, and coping behavior impairments, indicative of a skin-to-brain signaling axis. While a brain-skin axis has been described decades ago, initial descriptions were unidirectional, with the skin being the target of brain-initiated neuro-immune signaling, contributing to the pathogenesis of allergic and inflammatory skin diseases such as atopic dermatitis or psoriasis (Paus et al., 2006; Buske-Kirschbaum and Hellhammer, 2003; Buske-Kirschbaum et al., 1998). Only more recently has the concept expanded into bi-directional communication along this axis, with demonstration
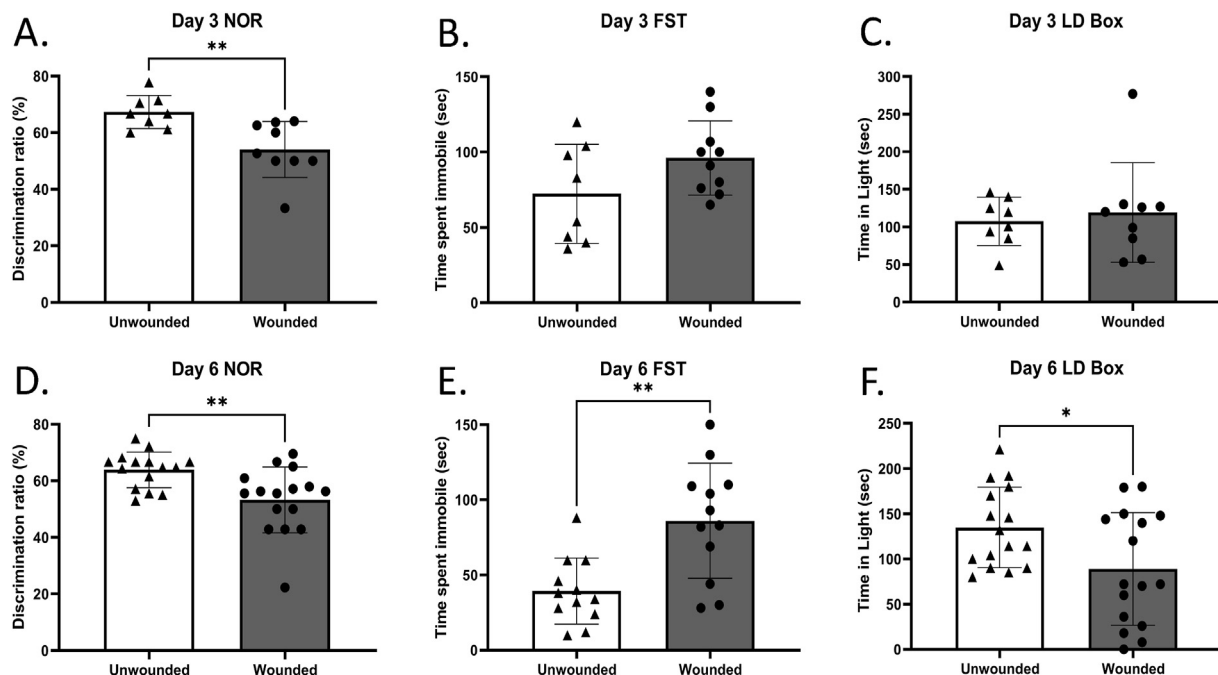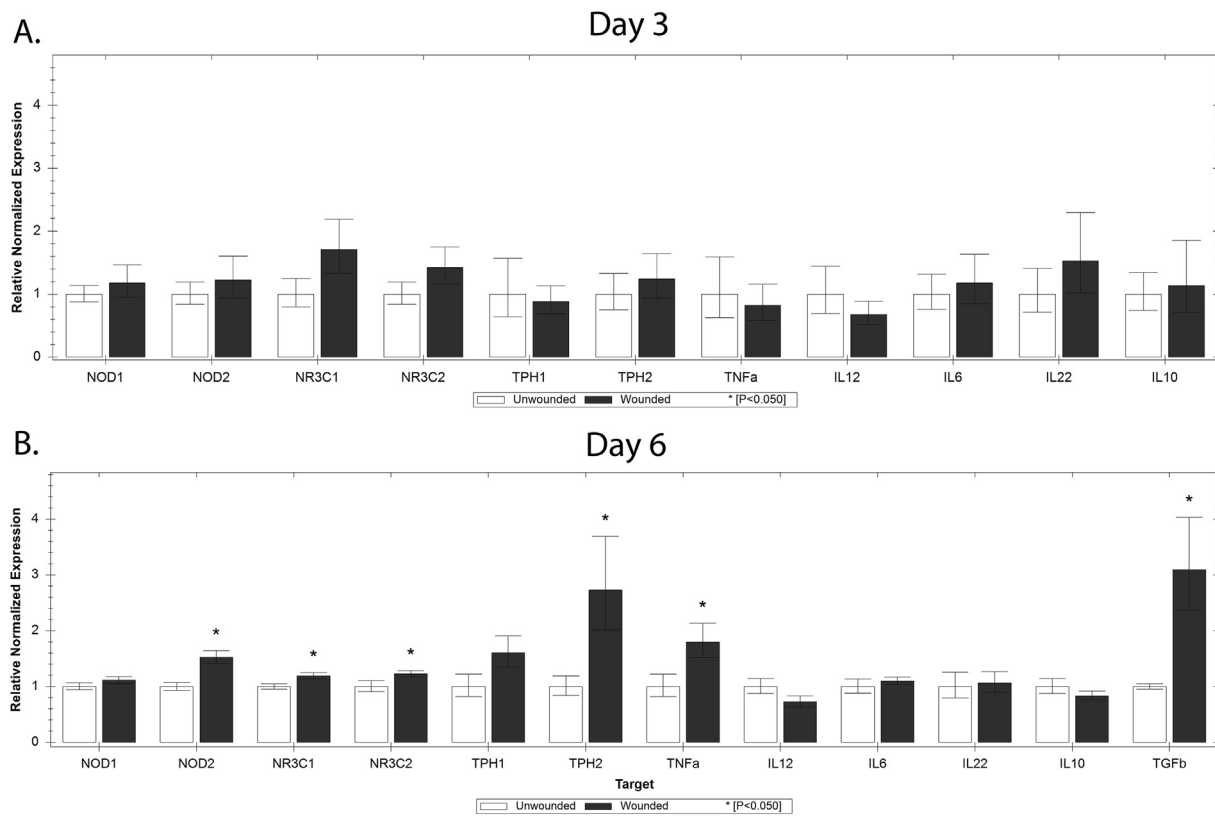


**Fig. 1.** Wounding results in behavioral deficits.
Animals were either wounded or sham-wounded, and at day 3 (A- C) or 6 (D-F) post-wounding were examined using the novel object recognition (NOR) test (A, D), the forced swim test (FST) (B, E), and the light/dark (L/D) test (C, F). n = 8–10 (Day 3) n = 16 (Day 6). Data are presented as the mean ± SEM, Unpaired, Non Parametric Mann-Whitey test *P < 0.05.

**Fig. 2. Expression of mediators of inflammation in the hippocampus of wounded and unwounded animals.**
Gene expression of NOD like receptors 1&2 (Nod1/Nod2), glucocorticoid and mineralcorticoid receptors (Nr3c1/Nr3c2), tryptophan hydroxylase (Tph1/Tph2), tumor necrosis factor alpha (Tnfa), and interleukins 12, 6, 22, and 10 (IL12, IL6, IL22, IL10) 3 days after wounding (Panel A, n = 5 unwounded, 10 wounded), and at day 6 (Panel B, n = 8 unwounded, 16 wounded). Data are presented as relative expression normalized to reference genes ±SEM, *P < 0.05.
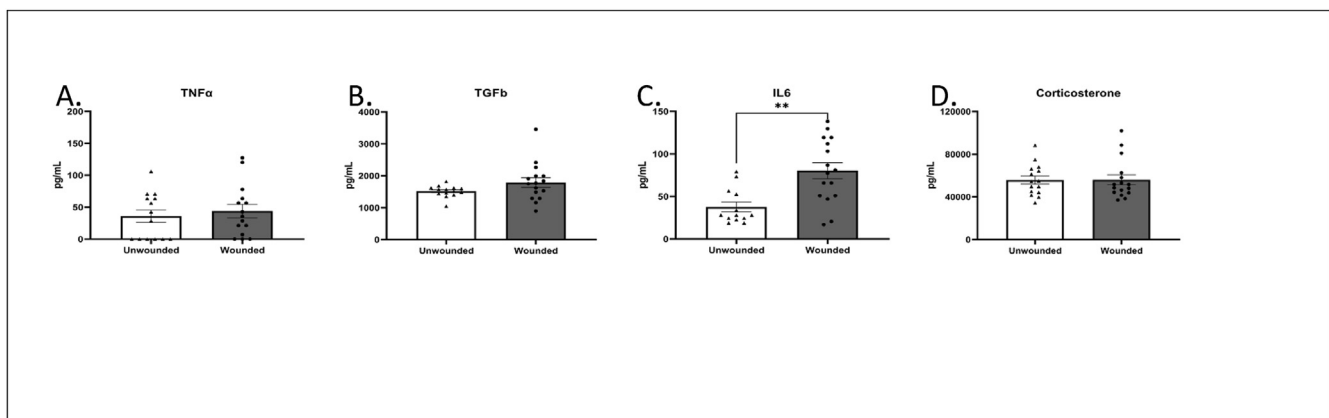


**Fig. 3.** Serum Inflammatory cytokines and corticosterone.
Serum concentrations of TNFα, TGFβ, IL6, and corticosterone 6 days after sham wounding or wounding (n = 15 unwounded, 16 wounded). Data are presented as mean concentration ±SEM, *P < 0.05, Unpaired Mann-Whitney test.

that peripheral, skin-resident nociceptors, when activated by microbial ligands or other noxious stimuli like UV irradiation, can generate signals that are delivered by ascending nerve routes to the brain with resultant neuroimmune responses (Cohen et al., 2019; Slominski et al., 2012; Yang and Chiu, 2017). Here we expand the paradigm of skin-brain bi-directional communication by demonstrating that trauma at the periphery, in the form of excisional wounding of the skin, can induce molecular changes in the brain, and importantly, that these are associated with cognitive and behavioral impairments. Thus, skin wounding is a newly identified noxious stimulus in the periphery that can initiate a skin-brain

reflex arc, resulting in behavioral changes.

Behavioral changes and cognitive deficits are common in patients with skin wounds of multiple etiologies, including, venous, arterial, pressure and diabetic foot ulcers, surgical wounds, and other traumatic wounds (Green et al., 2014; Kouris et al., 2016; Moffatt et al., 2009; Natovich et al., 2016; Platsidaki et al., 2017; Renner and Erfurt-Berge, 2017; Souza Nogueira et al., 2009; Udovichenko et al., 2017; Zhou and Jia, 2016; Coelho et al., 2014; Bui et al., 2018; Finlayson et al., 2017; Pedras et al., 2018). Approximately 40% of patients with diabetic skin ulcers exhibit depression (Udovichenko et al., 2017; Pedras et al., 2018),
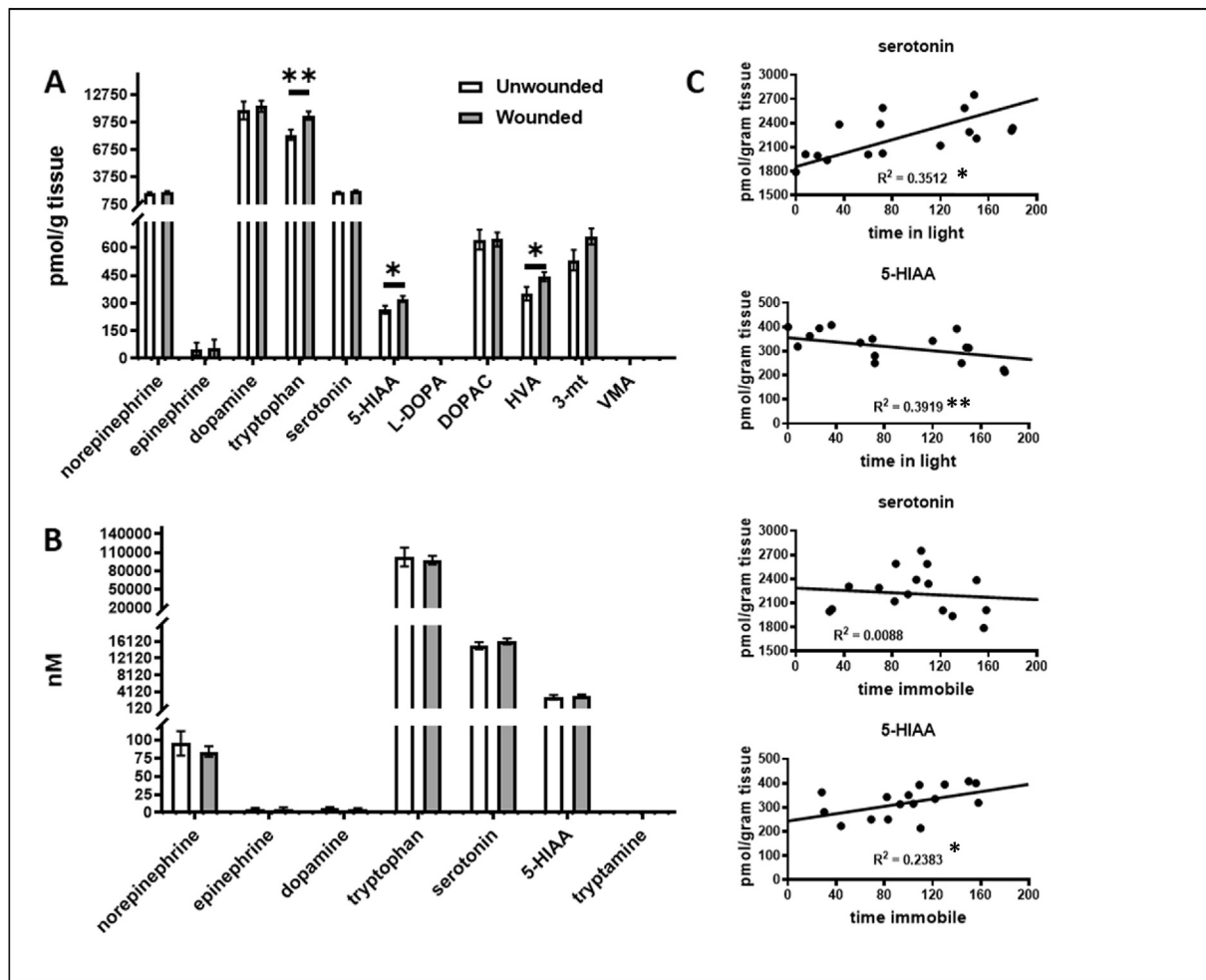
**Fig. 4. Cerebral cortex and serum catecholamines in wounded and unwounded animals.**
Cerebral cortices of wounded animals on day 6 post wounding demonstrate elevations in dopamine and serotonin pathway members (Panel A). No significant changes in levels of catecholamine neurochemicals noted in sera from wounded vs unwounded animals (Panel B) (n = 15 unwounded, 16 wounded). Data are presented as mean concentration ±SEM, *P < 0.05, Student's t-test for heteroscedastic data.
Linear correlations of cerebral cortex serotonin and its 5HIAA metabolite in the wounded animals with behavioral outcomes of the L/D and FST tests (Panel C). $R^2$ is the correlation coefficient. *P ≤ 0.050, **P < 0.01 ≤ 0.01.

and lower cognitive scores in multiple domains including episodic memory, attention and executive function (Natovich et al., 2016). Likewise there is a 30–40% prevalence of mood disorders in patients with venous ulcers (Souza Nogueira et al., 2009). These associations do not, however, define a causal relationship between the skin ulcer and the observed behavioral change. The current study supports a causal mechanism by demonstrating the association of alterations in brain signaling that could contribute to the observed altered behavior.

The observed associations in expression of hippocampal neuroinflammatory mediators in response to wounding suggest a potential mechanistic link. This is significant because of the implication of hippocampal involvement in neuroplasticity, behavior, and cognition (McKim et al., 2016). The wound-induced increase in hippocampal Tnfa is a hallmark of neuroinflammation, which has been associated with depression, and anxiety-like behavior in rodents (Guo et al., 2020; Wang et al., 2020; Zhang et al., 2020). Similarly, increased Tph2 mRNA (encoding tryptophan hydroxylase 2, the rate-limiting enzyme for brain serotonin, 5-HT biosynthesis) is observed in the dorsal raphe nucleus of rodents exhibiting increased anxiety-like behavior (Donner et al., 2012). We have previously demonstrated an increase in Nod1/2 expression in the hippocampus of adult mice treated with the colitogenic agent dextran sodium sulfate at weaning, which was also associated with cognitive impairments. This hippocampal PRR gene expression was coupled with

increased microglia activation and increased cytokine expression suggesting the presence of neuroinflammation (Salvo et al., 2020). Binding of glucocorticoids to the GR is important in sensitizing initial inflammatory responses by the innate immune system, resulting in IL6 production in macrophages *in vitro* (Busillo et al., 2011). Hippocampal increase in Tgfb expression is reported in association with multiple forms of brain injury (Villapol et al., 2013), and here the novel finding is its increased expression in the brain in response to injury in the periphery, even in the absence of concomitant increase in the serum. Since overexpression of Tgfb in the hippocampus has been shown to result in learning deficits in mice (Martinez-Canabal et al., 2013), it is reasonable to propose that likewise, the wound-induced increase in hippocampal Tgfb potentially contributes to the observed impairment of recognition memory observed in the wounded animals.

Other potential mechanistic links are evident in the observed alteration of the serotonergic and dopaminergic systems in the prefrontal cortex in the wounded animals relative to the controls. In this study we show that wounded mice perform worse on tasks assessing learning (NOR) anxiety-like behaviors (L/D box) and behavioral despair (FST). Wounded mice also had higher levels of metabolites (HVA and 5-HIAA) associated with elevated turnover of the CNS monoamines serotonin and dopamine. CNS dopamine is important for motivation, learning (Cox and Witten, 2019) and memory consolidation (Wise, 2004) which may

account for poorer performance in wounded mice on novel learning and behavioral tasks. The L/D box test is an important model for anxiety-like behavior (Bourin and Hascoet, 2003), while antidepressants that modulate serotonin have been shown to improve performance (De Angelis and Furlan, 2000; Hascoet et al., 2000). Among wounded mice, improved performance on L/D box test was associated with higher levels of cerebral serotonin and reduced levels of serotonin breakdown as measured by 5-HIAA. These findings align with monoamine models of mood disorders where serotonin depletion is associated with anxiety (Booij et al., 2003). The FST is a behavioral model to assess coping following an inescapable stress and previously associated with depressive behaviors (Cryan and Holmes, 2005). Performance in the FST is also improved by antidepressants that modulate CNS monoamines (Petit-Demouliere et al., 2005). Among wounded mice, higher levels of 5-HIAA trended towards significance in being associated with worse performance. The monoamine hypothesis of depression continues to inform the most effective treatments for mood disorders that work to modulate and increase CNS serotonin, dopamine and other amine neurotransmitters, transporters and receptors (Jans et al., 2007; Hillhouse and Porter, 2015). Future translational research could consider the utility of common antidepressants that modulate serotonin and dopamine in targeting negative mood symptoms and behaviors among patients with chronic wounds to promote wound healing.

Systemic elevation of IL6 was also observed in day 6 wounded animals. IL6 expression is implicated in pleiotropic responses in cutaneous wounds. In the early phase post-wounding it signals for pro-inflammatory responses to injury, recruiting cytokines and immune cells, and in later phases, IL6 shifts to induce pro-reparative and fibrogenic responses in the anti-inflammatory M2 polarized macrophages, supporting resolution of wound healing. (Johnson et al., 2020), (Bosurgi et al., 2017; Braune et al., 2017), (Wood et al., 2011) Given our findings that serum IL6 was increased at 6 days post-wounding, this would suggest it is involved in regulating wound resolution. There is also extensive documentation of strong correlation of elevated IL6 serum levels in patients with major depressive disorder (Hadian et al., 2020; Roohi et al., 2021). While the exact underpinnings of this association are not completely understood, one documented action of IL6 is the control of the level of the serotonin transporter, and subsequently serotonin uptake (Kong et al., 2015). Thus, the noted increase in systemic levels of IL6 post-wounding could account for the observed increased dopaminergic and serotonergic turnover in the frontal cortex in wounded animals. It is conceivable then, that wound-induced IL6 elevations in the serum influence neurotransmitter metabolism resulting in the observed behavioral changes.

There are some limitations to the current study. Female mice were used to avoid confounding factors that may be attributed sexual dimorphism in wound healing (Gilliver et al., 2008). Thus, future studies should aim to determine if there are sex-based changes in behavior in response to wounding by including male animals. Extended periods of post-wounding evaluation will be needed to determine how long the cognitive alterations persist. The wounds examined in this study do not model human chronic non-healing wounds, since they do heal over time. No one animal model fully reproduces human chronic wounds, to which aging, diabetes, ischemia, immune dysfunction and infection can contribute (Ansell et al., 2012). Future studies will address each of these contributors to wound chronicity and how that may alter cognition. Other factors like bacterial infection in the wound may exacerbate the behavioral changes, much like gut dysbiosis affects cognition and behavior. It is difficult to isolate the possible contribution of pain to the observed behavior results, as pain-mitigating therapeutic approaches induce their own central CNS alterations. Finally, additional studies are needed to fully elucidate the signaling pathway from skin to spinal cord to brain to determine if it is mediated by systemic inflammation and elevation of blood levels of IL6 or a direct signaling pathway from peripheral nociceptors to the CNS.

### 4.1. Implications for clinical translation

Depression and cognitive impairment are associated with decreased treatment compliance and adherence to self-care tasks (Feil et al., 2012; Gonzalez et al., 2008; Lin et al., 2004; Pearson et al., 2014). Treatment compliance and persistent self-care are critical for optimal wound healing. Patients need to understand how to properly manage their wounds while following recommendations regarding changes in medications and lifestyle habits. When patients are proactively engaged in their treatment, outcomes are improved (Vileikyte et al., 2009). Thus, understanding the pathogenesis of the associated cognitive deficits and depressive behaviors in chronic wound patients can potentially improve their wound outcomes.

### 5. Conclusion

Skin wounding results in behavioral aberrations, including cognitive impairments, anxiety-like behavior and coping deficits, coupled with associated molecular changes in the brain. These behavioral deficits may indicate a directional skin-to-brain signaling pathway, or an indirect mechanism, stemming from wound-induced elevations of systemic IL6. In either case, these findings expand the current paradigm of bi-directional signaling along this axis. This new understanding of how skin wounds may detrimentally affect the brain and behavior, may provide novel insights on potential targets to disrupt the dysregulation of the skin-brain axis following wounding.

### Author contributions

DRF: conducting experiments, acquiring data, analyzing data.
YH: conducting experiments, acquiring data.
ACG: acquiring data, analyzing data.
DD: conducting experiments.
JM: acquiring data.
IK: writing the manuscript.
MGG: conceiving and designing the analysis, writing the manuscript.
RRI: conceiving and designing the analysis, writing the manuscript.

### Declaration of competing interest

The Authors declare that there is no conflict of interest.

### Acknowledgements

### Appendix A. Supplementary data

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