

Chronic Psychological Distress as an Inducer of Microglial Activation and Leukocyte Recruitment into the Area Postrema

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Key Words

Area postrema · CD11b · CD45 · Leukocyte recruitment · Lymphocytes · Microglial activation · Monocytes · Neuroinflammation · TrkA

Abstract

Background: Chronic psychological distress can cause neuroinflammation, but the involvement of leukocytes in this inflammatory response remains unclear. The area postrema (AP) is considered a neural-immune interface because it lacks a blood-brain barrier and a site for leukocyte recruitment in neuroinflammatory conditions induced by immunological insults, but its role in chronic psychological distress has not been explored. **Objective:** To determine leukocyte recruitment to the AP after chronic psychological distress. **Methods:** Rats were exposed to cat odor for 5 consecutive days to induce distress, and, on the 6th day, their brains were dissected to perform immunohistofluorescence studies of the AP. Immune cells were identified and quantified with CD45 and CD11b markers. The distribution of neurons and immune cells was determined using TrkA and CD45 markers, respectively. **Results:** Distress induced a significant increase in CD45⁺ and CD11b⁺ cells in the AP. Three immunophenotypes were determined in the control and distress groups: CD45⁺/CD11b⁻, CD45⁺/CD11b⁺ and CD45⁻/CD11b⁺. CD expression, morphology and fluorescence intensity enabled

the identification of different immune cell types: starting from longitudinal ramified microglia (mainly in the control group) to amoeboid microglia, monocytes and lymphocytes (mostly in the distressed group). TrkA and CD45 expression in the AP revealed the proximity between soma neurons and leukocytes. Interestingly, some CD45⁺ cells expressed TrkA, with increased expression in the distressed group. **Conclusions:** The identification of microglial activation, leukocyte recruitment and the close proximity between neurons and leukocytes in the AP after chronic psychological distress exposure suggests the AP as a site for distress-induced immune responses and engraftment of leukocytes infiltrating the CNS.

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Introduction

Chronic psychological distress induces inflammatory responses in the central nervous system (CNS) and, consequently, several neurological diseases. Thus, the nervous system and the immune system maintain extensive communication. This process has been mainly studied through the activity of pro- and anti-inflammatory molecules [1–3]. One of the major defenses against neuroinflammation induced by chronic psychological distress are microglia, which prevent neuronal damage, but pro-

longed microglial activation can be harmful to brain function and behavior [4]. In addition, chronic distress increases the number of microglia in certain stress-sensitive brain regions, and microglia shift markedly from a ramified-resting state to a nonresting state [5].

Nevertheless, microglia cannot provide all the necessary protection and recovery from CNS injury and disease. Therefore, monocyte-derived macrophage recruitment is required for assistance in the immunization of resident cells exerting different activities in order to repair damaged CNS [6]. Few works have described the role of myeloid-derived cells recruited from the blood to the CNS under psychological distress conditions. Essentially, monocyte recruitment to the brain was found to be increased in response to social stress, which indicated an association of this event with the development of anxiety-like behavior [7, 8].

The sensory circumventricular organ (CVO), the area postrema (AP), is described as a blood-CNS interface because it lacks blood-brain barrier (BBB) [9]. The AP has a vast fenestrated vasculature and neurons that sense physicochemical conditions from the circulating blood, such as osmotic pressure, cardiovascular tone, immune activation and metabolic status. Located in the fourth ventricle, it also provides and receives inputs from a variety of autonomic centers [10, 11]. Our attention was focused on the AP as a route of entry for proinflammatory molecules [12]. Moreover, the AP, as well as other CVOs, has been characterized as a site for immune cell recruitment restricted to the presence of immunogens, such as bacterial lipopolysaccharides (LPS) [13, 14], and experimental autoimmune encephalomyelitis (EAE) [15, 16].

Due to the limited data on the AP following psychological distress, we performed this study. During stress, the hypothalamic-hypophyseal-adrenal axis is regulated by cytokines acting on the vagus, which, in turn, communicates with the AP [17]. Recently, increased metabolic activity was noted in the AP following exposure to a psychosocial stressor [18].

Taken together, based on the evidence that chronic psychological distress causes neuroinflammation and that the AP is a structure that recruits leukocytes in response to immunological stimuli during a neuroinflammatory process, our objective was to test if leukocyte recruitment to the AP is induced by chronic psychological distress and to demonstrate if chronic psychological distress induces an increase in the number of AP immune cells, i.e. activation of microglia and leukocytes, close proximity between neurons and leukocytes and expression of neuronal molecules on immune cells.

Materials and Methods

Animals

All procedures were conducted in accordance with the international standards of the Institutional Bioethical Committee. Male Sprague-Dawley rats were born and raised at the Biomedical Science Institute vivarium of the Universidad Autónoma de Ciudad Juárez. The light and dark cycle lasted 12 h (lights turned on at 7 a.m.), respectively, and food and water were available ad libitum. From birth up until 1 month of age, pups were maternally nursed; thereafter, during the next 15 days, males were kept together. Two weeks before the experimental procedures, all males were housed singly until they reached a weight of 230–280 g.

Exposure to Distress

The cat odor exposure method was used as psychological-induced distress. Each rat was placed briefly in one of two individual avoidance chambers. In the first chamber, rats were kept for a 20-min habituation; thereafter, the animal was placed back into the housing cage for another 20 min to relax. Then, the animal was again transferred for 20 min to the avoidance chamber, which contained either a normal piece (25 × 25 cm) of cotton fabric without cat odor (control) or one impregnated with cat odor (a carpet used by an adult cat) to induce distress. This experimental procedure was repeated daily from 8.00 to 9.00 a.m. for 5 days [19, 20].

Immunohistofluorescence

On the 6th day, rats were sacrificed using a lethal dose of sodium pentobarbital (380 mg/kg i.p.). Transcardial perfusion was performed through the left ventricle with PBS (pH 7.4), and the right atrium was opened and perfused for 10 min at 10 ml/min, followed by another 20-min perfusion containing 4% paraformaldehyde performed at the same rate with PBS (pH 7.4). Brains were dissected and equilibrated in 50 ml of 30% sucrose at 4°C until they were denser than the sucrose solution (around 48 h). Then they were embedded in OCT and frozen at –25°C to obtain 10- μ m coronal sections using a cryostat (Leica CM1510-S). The AP structure was localized between –13.68 and –14.16 mm from bregma using the stereotaxic atlas of Paxinos and Watson [21]. Sections were collected and mounted on precoated 1% gelatin slides. All brain tissue sections were maintained at –30°C until use. For CD45 and CD11b double labeling, sections were blocked for 30 min in PBS containing 5% BSA and then incubated at room temperature for 2 h with a primary antibody mix consisting of 1:60 mouse anti-rat CD45 (ab33923; abcam) and 1:300 rabbit anti-rat CD11b (ab75476; abcam) antibodies in PBS (pH 7.4) containing 1% BSA. Sections were washed in PBS three times for 10 min each; secondary antibody incubation was carried out using 1:2,000 goat anti-mouse Alexa Fluor 488 (A11001; Invitrogen) and 1:2,000 goat anti-rabbit Alexa Fluor 594 (A11037; Invitrogen) mix in PBS (pH 7.4) containing 1% BSA; thereafter, sections were washed three times in PBS for 10 min each, with the last wash being done for 5 min with deionized water alone. Finally, a mounting medium (Fluoroshield; Sigma) with added nucleic acid stain, 4',6-diamidino-2-phenylindole dihydrochloride (DAPI), was used, and then sections were coverslipped and frozen at –30°C or immediately visualized on a high-performance fluorescence microscope (Leica DM2000). For double-labeling of CD45 and TrkA sections, the above-described protocol was followed, but the primary antibody mix was 1:60 mouse anti-rat CD45 (ab33923; abcam) and 1:400 rabbit anti-rat TrkA (ab8871; abcam); secondary antibodies and their dilutions were as described above.

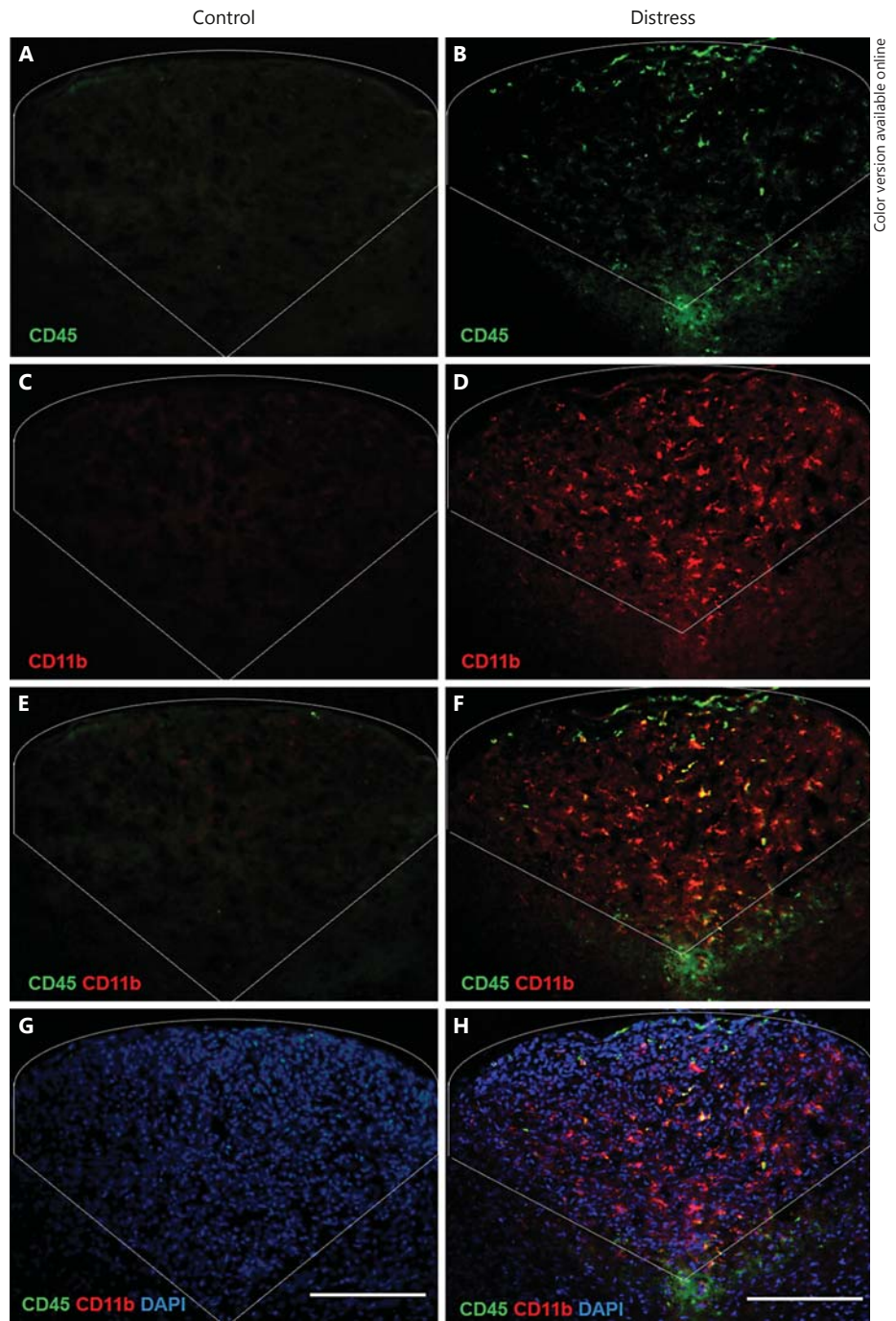


Fig. 1. Representative images showing coronal immunostaining of AP sections (10 μm thick) of leukocyte common antigen CD45 (green, Alexa Fluor 488), macrophage-1 antigen CD11b (red, Alexa Fluor 594) and nucleus (blue, DAPI; **G, H**; see online version for colors). Photomicrographs are settled to observe differences in CD45 (**A, B, E–H**) and CD11b (**C–H**) expression among control (**A, C, E, G**) and distressed rats (**B, D, F, H**). The AP is delimited by a dashed line. Scale bars = 100 μm .

Cell Quantification and Statistical Data Analysis

Positive cells were counted on 3 consecutive coronal sections from the frontal zone, which represents the main AP volume (bregma -13.68 to -13.8 mm), and means were obtained from each rat. Images were taken with a camera (Leica DFC420C) coupled to a high-performance fluorescence microscope (Leica DM2000) and processed with Leica application suite Microsystems software (version 3.1.0). All fluorescent signals from the tissue were con-

firmed as cells by their localization determined by DAPI-stained nuclei. Cells were counted in a masked fashion considering the AP only. The kind of CD expression was determined either as single or double. We refer to the kind of admixture of CDs as immunophenotypes; the immunomorphophenotype refers to the whole of the characteristics: admixture of CDs, intensity of their expression and cell morphology.

In double labeling for TrkA and CD45 assays, the relative TrkA⁺/CD45⁺ expression [TrkA/CD45 (%)] was obtained as an equation:

$$\text{TrkA/CD45 (\%)} = \frac{[\text{TrkA}^+/\text{CD45}^+]}{[\text{TrkA}^+/\text{CD45}^+] + [\text{TrkA}^-/\text{CD45}^+]} \times 100.$$

Student's t test was used to compare differences between the distress group and the control group. p values <5% were deemed significant. All data are expressed as means ± SE.

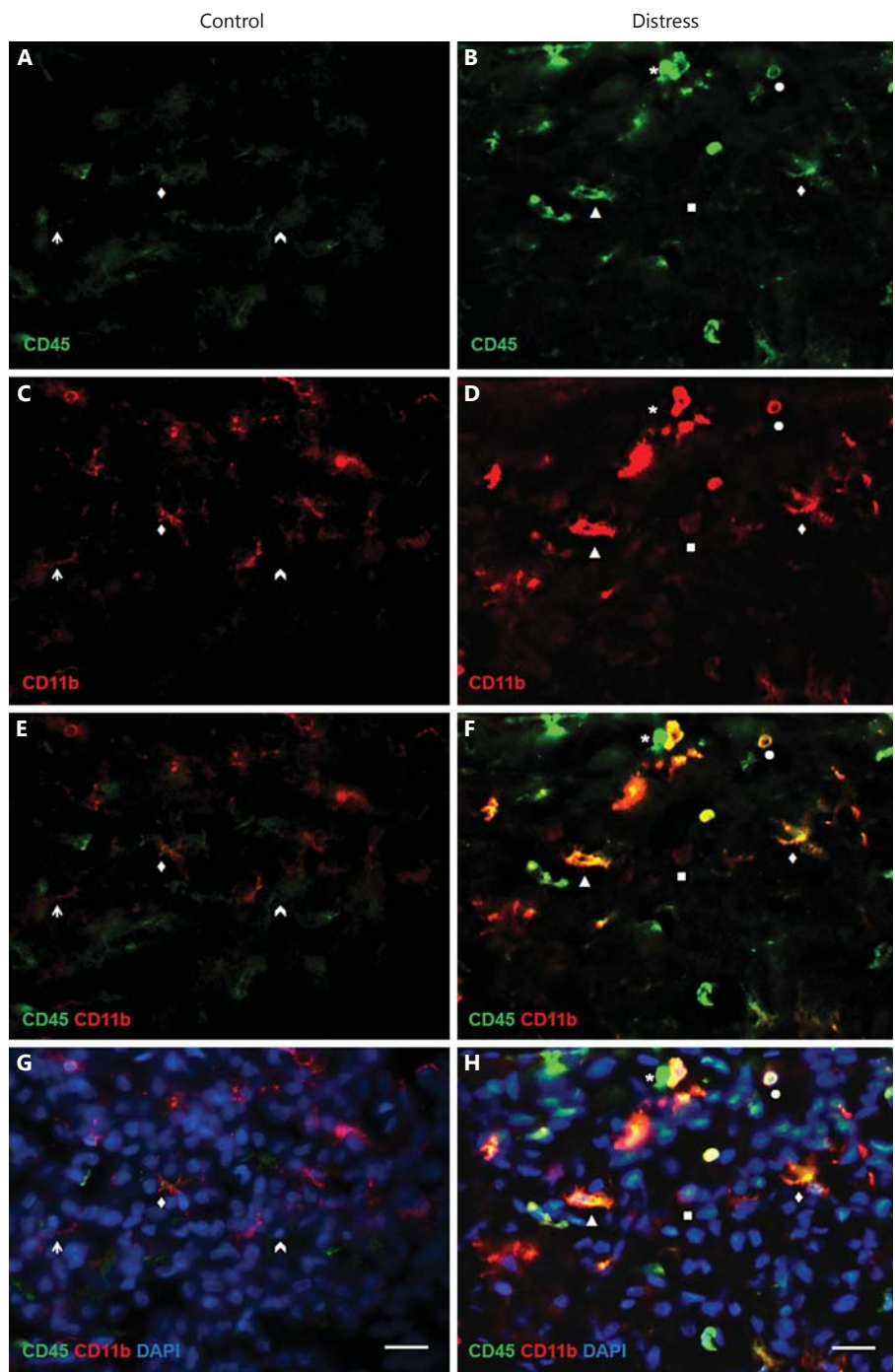


Fig. 2. Immunomorphological description of immune cells in 2 representative AP coronal sections (10 μm thick) from a control and a distressed rat. Immunostaining of leukocyte common antigen CD45 (A, B; Alexa Fluor 488); macrophage-1 antigen CD11b (C, D; Alexa Fluor 594); merged fluorescence of CD45 and CD11b (E, F), and merged fluorescence of CD45, CD11b and DAPI (G, H). Control group: ^ = CD45⁺/CD11b⁻ ramified microglia; ◆ = CD45⁺/CD11b⁺ ramified microglia; † = CD45⁻/CD11b⁺ ramified microglia; distress group: * = CD45⁺/CD11b⁻ round lymphocyte-like cell; ● = CD45⁺/CD11b⁺ monocyte-like cell; ▲ = CD45⁺/CD11b⁺ amoeboid microglia; ◆ = CD45⁺/CD11b⁺ macrophage-like cell or ramified microglia; ■ = CD45⁻/CD11b⁺ amoeboid microglia. Scale bars = 20 μm.

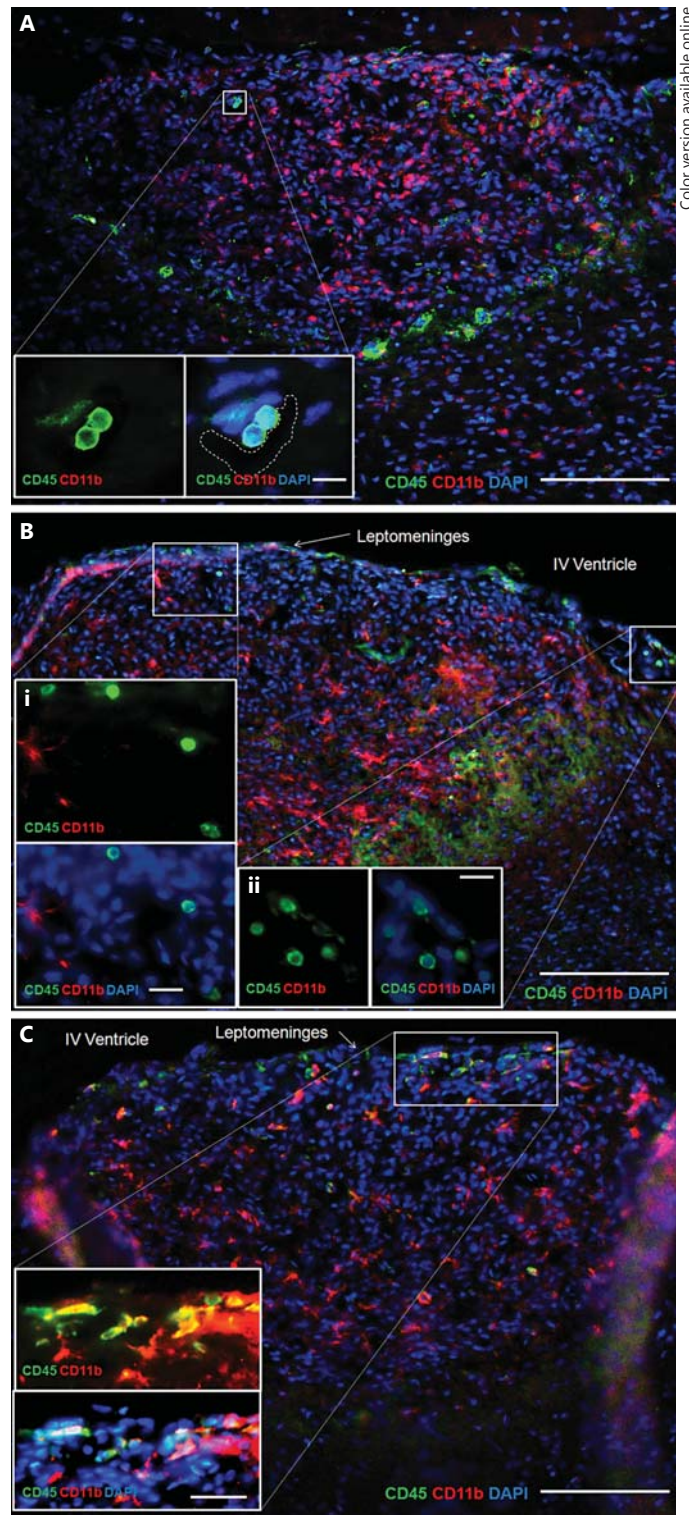
Results

Classification and Distribution of CD45⁺ and CD11b⁺ AP Cells

To examine the population of immune AP cells by double immunohistochemistry for CD45 and CD11b, 3 consecutive coronal AP sections of each brain from control and distressed rats were used (n = 7 and n = 9, respectively). Positive cells were classified according to marker expression and morphology. Figure 1 depicts representative images of the significant increases in fluorescence intensity and numbers of CD45⁺ and CD11b⁺ cells in the AP of distressed animals in contrast to the control group (fig. 1B, D, F, H vs. 1A, C, E, G).

Three immunophenotypes were found in both groups: CD45⁺/CD11b⁻, CD45⁺/CD11b⁺ and CD45⁻/CD11b⁺. Moreover, differences in fluorescence intensity and morphology of the immune cells were also detected between the groups (fig. 2). In the control animals, nearly all of the observed cells were longitudinal ramified microglia expressing one or both markers [fig. 2: CD45⁺/CD11b⁻ (arrowhead), CD45⁺/CD11b⁺ (diamond) and CD45⁻/CD11b⁺ (arrow in)]. Immune cells in the distress group presented the three immunophenotypes described as well as longitudinal ramified microglia; moreover, new morphological changes appeared concomitant with increased fluorescence intensity. One of the morphological changes observed in the distress group was loss of ramifications in microglial cells (fig. 2), shifting their shapes to amoeboid-like cells with CD45⁺/CD11b⁺ (triangle) and CD45⁻/CD11b⁺ immunophenotypes (square). This last immunomorphophenotype was the most abundant in the distress group. The CD45⁺/CD11b⁺ immunophenotype, classified as ramified microglia in the control group, was conserved, but in the distress condition higher fluores-

Fig. 3. Representative images of coronal immunostaining of AP sections (10 μ m thick) from distressed rats. Immunostaining of leukocyte common antigen CD45 (green, Alexa Fluor 488), macrophage-1 antigen CD11b (red, Alexa Fluor 594) and nucleus (blue, DAPI; see online version for colors). **A** Two CD45⁺/CD11b⁻ lymphocytes next to a CD45⁺/CD11b⁻ ramified microglial cell; dashed line inside the inset indicates the lumen of a vessel. **Bi** CD45⁺/CD11b⁻ round lymphocytes in the dorsal periventricular mantle zone next to meningeal tissue; **Bii** CD45⁺/CD11b⁻ round lymphocytes in meningeal tissue. **C** CD45⁺/CD11b⁺ monocytes accumulate between the border of transitional AP to meningeal tissue and the IVth ventricle. Scale bars = 100 μ m (insets = 10, 15 and 20 μ m, respectively). The panels with the scale bars also show DAPI staining.



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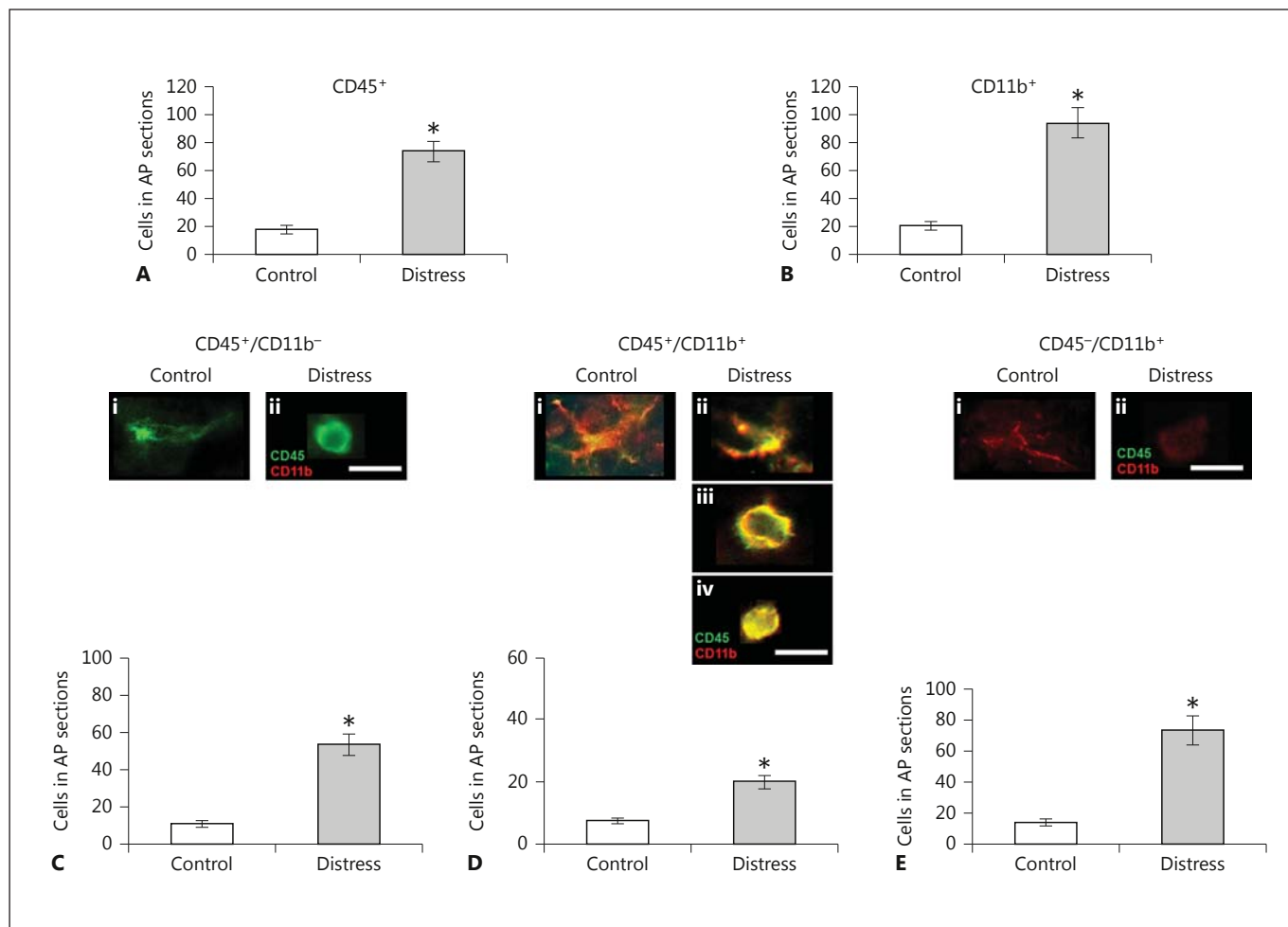


Fig. 4. Cell numbers of CD45⁺ (**A**) and CD11b⁺ (**B**) in AP coronal sections (10 μ m thick). Morphological changes are shown for each immunophenotype. **C** Immunomorphophenotypes of CD45⁺/CD11b⁻ cells (see online version for colors): longitudinal ramified microglia (**i**) and lymphocyte (**ii**). **D** Immunomorphophenotypes of CD45⁺/CD11b⁺ cells (see online version for colors): longitudi-

nal ramified microglia (**i**); macrophage-like cell or reactive ramified microglia (**ii**); amoeboid microglia (**iii**) and monocyte (**iv**). **E** Immunomorphophenotypes of CD45⁻/CD11b⁺ cells: longitudinal ramified microglia (**i**) and amoeboid microglia (**ii**). $n = 7-9$, * $p < 0.001$. Scale bars = 10 μ m.

cence intensity was noted, suggesting the immunomorphophenotype of reactive microglia with retraction of branches or macrophages (fig. 2: diamond). In figure 2, lymphocytes (round, 7 μ m) appeared with CD45⁺/CD11b⁻ immunophenotype (asterisk) and monocytes (ovoid, 10 μ m) as CD45⁺/CD11b⁺ immunophenotype (circle), both with high fluorescence intensity. It should be emphasized that these last two immunomorphophenotypes were practically absent in the control group (fig. 1, 2).

In the distress group, interesting events were observed regarding the distribution of leukocytes in all the differ-

ent AP structures, i.e. vessels, dorsal periventricular mantle zone and leptomeninges. Lymphocytes or monocytes were localized inside the lumen of a vessel (fig. 3A). Moreover, lymphocytes (fig. 3B) and elongated monocytes (fig. 3C) were noted in the dorsal periventricular mantle zone of the AP near leptomeninges or meningeal tissue.

Quantification of CD45⁺ and CD11b⁺ Cells in the AP

Quantification of CD45⁺ and CD11b⁺ cells and the three immunophenotypes (CD45⁺/CD11b⁻, CD45⁺/CD11b⁺ and CD45⁻/CD11b⁺) inside the AP was carried

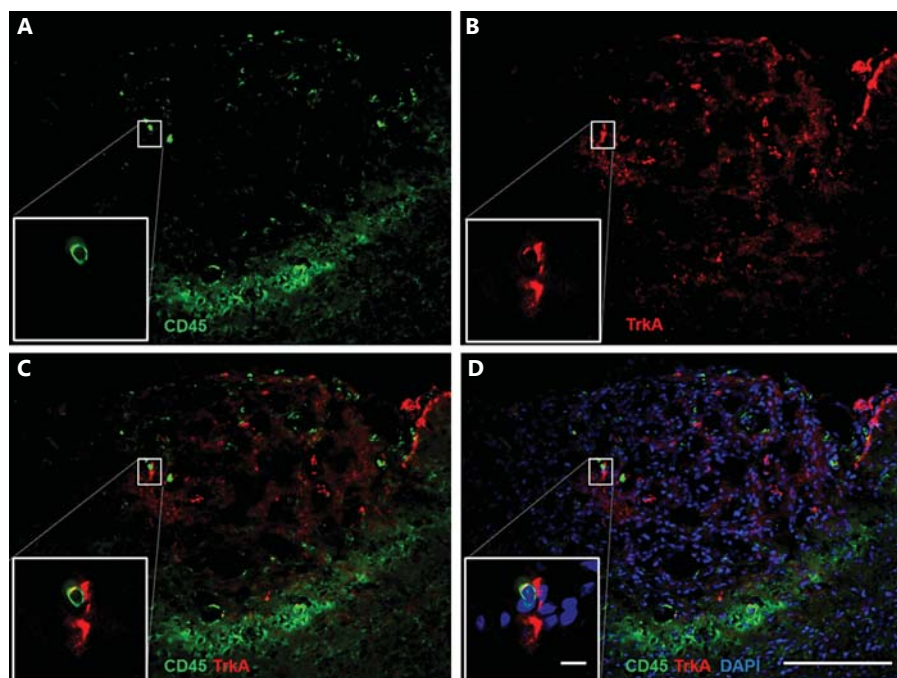


Fig. 5. Representative images showing a neuronal marker (TrkA) and an immune cell marker (CD45) in AP sections (10 μm thick) of a distressed rat (see online version for colors). **A, B** Immunostaining of CD45 (**A**; green, Alexa Fluor 488) and TrkA (**B**; red, Alexa Fluor 594). **C** Merged image of TrkA and CD45 fluorescence. **D** Merged image of TrkA, CD45 and nucleus (blue DAPI). **Insets** Magnifications showing the contact between a CD45⁺ round cell (leukocyte) and a TrkA⁺ larger cell (soma of the neuron). Scale bars = 100 μm (insets = 10 μm).

out without considering morphology, fluorescence intensity or size. Nevertheless, a brief classification of the most abundant cells for each immunomorphophenotype, both for control and distress groups, is given in figure 4. The average total CD45⁺ cell number increased four times in the distress condition [18 ± 2 (control) vs. 74 ± 7 (distress), $p < 0.001$]; in contrast, CD11b⁺ cells were nearly 30% more abundant than CD45⁺ cells and also increased nearly five times in the distress condition [21 ± 3 (control) vs. 94 ± 11 (distress), $p < 0.001$]. All three given immunophenotypes (CD45⁺/CD11b⁻, CD45⁺/CD11b⁺ and CD45⁻/CD11b⁺) also increased six [11 ± 2 (control) vs. 53 ± 6 (distress), $p < 0.001$], three [7 ± 1 (control) vs. 21 ± 2 (distress), $p < 0.001$] and seven times [14 ± 2 (control) vs. 73 ± 10 (distress), $p < 0.001$], respectively (fig. 4).

Detection of TrkA⁺ and CD45⁺ Cells in the AP

TrkA and CD45 markers were used to perform double immunohistofluorescence in coronal AP sections in order to detect neurons and immune cells, respectively (fig. 5A, B). In some of the sections analyzed, it was possible to observe CD45⁺ leukocytes making close contact with TrkA⁺ soma neurons (see insets in fig. 5C, D). Another interesting phenomenon was that microglia (fig. 6i) and leukocytes (fig. 6ii) were able to express CD45 as well as TrkA in the AP. Statistical analysis of this coexpression was carried out in 7 rats from each group. The data dem-

onstrated that under distressed condition there was an increment in the relative expression of TrkA/CD45 [fig. 7: 8 ± 0 (control) vs. 13 ± 2 (distress), $p < 0.05$].

Discussion

The results of this study show that chronic psychological distress can lead to an increased number of CD45⁺ and CD11b⁺ cells in the AP (fig. 1, 4). These data demonstrate changes in microglia going from longitudinal (control) to amoeboid morphology (distress) as well as differences in marker expression, and, even more remarkably, evidence of leukocyte recruitment (fig. 2), e.g. lymphocytes (CD45⁺/CD11b⁻: round cells) and monocytes (CD45⁺/CD11b⁺: ovoid cells). Because this makes it possible to differentiate these leukocyte types by shape and fluorescent intensity from microglia [22–24], this evidence suggests leukocyte recruitment to the AP following psychological distress. We also suggest that these leukocytes are situated around vessels and in the dorsal periventricular mantle zone of the AP near leptomeningeal tissue (fig. 3) [25, 26]. Furthermore, we found very close proximity between leukocytes (CD45⁺) and neurons (TrkA⁺) inside the AP (fig. 5) resembling synapse-like stable interactions [27]. This physical contact between a CNS neuron and a leukocyte in a distress situation which

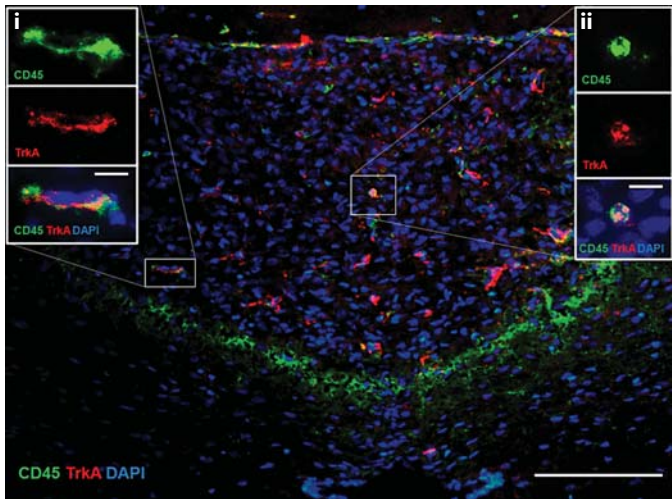


Fig. 6. Representative image showing coexpression of neuronal (TrkA; middle and lower insets) and immune cell markers (CD45; upper and lower insets) in AP sections (10 μ m thick) of a distressed rat: microglial cell (i) and circulating leukocyte (ii). Scale bars = 100 μ m (insets = 10 μ m). DAPI (blue; see online version for colors) is shown in the main and lower inset panels.

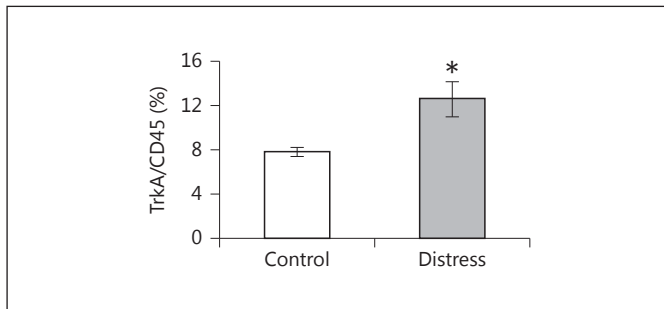


Fig. 7. Relative expression of TrkA⁺/CD45⁺ in AP cells. Means \pm SE, n = 7 for each group. * p < 0.05.

is not mediated by an infection is up to the knowledge of the authors observed for the first time. Also, we observed significantly increased TrkA⁺ expression by some CD45⁺ cells (microglia and leukocytes) under chronic psychological distress conditions (fig. 6, 7).

Evidence of Multiple Immunomorphophenotypes of CD45⁺ and CD11b⁺ Cells in the AP

It has been reported that the identification of certain immune cell populations in the brain could be difficult because of the varying morphology and expression of markers [14]. We used CD45 and CD11b surface markers, which are robustly expressed on leukocytes [28], but

microglia can also express these markers with less intensity than leukocytes [23]. In the AP, we classified three subpopulations of immunophenotypes considering CD profiles and their morphology (fig. 4). The CD45⁺/CD11b⁻ immunophenotype with high intensity and round shape was classified as any type of circulating immune cell such as lymphocytes and with low intensity as longitudinal ramified microglia (resting microglia); the CD45⁺/CD11b⁺ immunophenotype with high intensity was classified as monocytes, macrophages or reactive microglia/amoeboid (phagocytic) microglia and with low intensity as longitudinal ramified microglia (resting), and the CD45⁻/CD11b⁺ immunophenotype as amoeboid (phagocytic) microglia and longitudinal ramified microglia (resting microglia) [4, 22–24, 29].

However, the CD45⁺/CD11b⁺ cell subset found under chronic psychological distress, considered as monocytes and macrophages, is related to neuroinflammation in the CNS. These cell types contribute to inflammation enhancement [30] and arrive only when the CNS is damaged [31]. In EAE, depletion of monocytes entering the brain delays adverse effects of the condition showing that infiltrated monocytes/macrophages play a major role in the development of neurological disorders following neuronal tissue damage [32]. In *Streptococcus pneumoniae* infection-induced neuropathology, circulating monocytes are involved in the tissue damage because of the increased lysosomal activity in close proximity to apoptotic cells [33]. However, circulating monocytes (CD45⁺/CD11⁺) entering adult murine brain have been considered responsible for microglial repopulation [34]. For example, the recruitment of green fluorescent protein (GFP)-labeled bone marrow monocytes to the brain produces anxiety-like behavior after social stress exposure, and these cells were able to evolve into macrophages. Interestingly, the authors did not associate BBB permeability with the increase in macrophages [8]. These data lend support to our findings, suggesting the AP as a route of entry for immune blood cells into the brain. In other work using transplantation of GFP-labeled bone marrow cells, the migration of these cells into the CNS was demonstrated; nearly all of the infiltrated cells had a highly ramified morphology expressing microglial markers with the AP being a region with consistently high infiltration of GFP-positive cells [35]. Nowadays, there is a tendency to explain monocyte-macrophage CNS infiltration as a prerequisite for healing of damaged brain when activated microglia are less effective in damage control [36, 37]. These last three findings are consistent with our results in the AP under chronic psychological distress and may support

the idea that in damaged CNS, the AP is the site for monocyte recruitment in order to assist microglia in the repair process.

On the other hand, the high increase in CD45⁺ and CD11b⁺ cells in the AP, most of them with a microglial shape, is consistent with the work of Tynan et al. [5], who found increased density and activation of microglial cells in 15 stress-responsive brain regions following chronic stress exposure. The AP was not included in their evaluation, but our work suggests the AP as a distress immune-responsive structure. The classification of amoeboid or loss of ramification in CD45⁺/CD11b⁺ and CD45⁻/CD11b⁺ microglial cells in the AP under stress (fig. 2, 4) was mostly in accordance with Jurgens and Johnson [4]. They indicated that stress inflammation enhances microglial activation, which in turn produces neuronal damage/atrophy, i.e. the first step in the loss of ramification of glial activation (reactive microglia) followed by transformation into phagocytic cells with amoeboid appearance [38]. The microglial activation and regulation of circulating monocytes in the CNS is an important event in the mediation of depressive-like behavior [39].

The AP lying at the base of the fourth ventricle and the absence of the BBB make the AP a privileged route of entry for circulating components. As shown in figure 3A, CD45⁺/CD11b⁻ circulating lymphocytes present in the AP enter from the lumen through a characteristic fenestrated AP vessel that lacks the BBB [9]. Figure 3B shows that these CD45⁺/CD11b⁻ circulating lymphocytes accumulate between the dorsal periventricular mantle zone of the AP and meningeal tissue (fig. 3Bi, ii, respectively) as well as elongated CD45⁺/CD11b⁺ cells (fig. 3C) suggesting an exchange of lymphocytes and monocytes between meningeal tissue and the AP. The presence of CD45⁺/CD11b⁻ circulating lymphocytes in the AP could suggest a surveillance response of the immune system by lymphocytes, specifically T cells [40]. This evidence supports the idea of an exchange of cells between leptomeninges [41] and a possible entrance for leukocytes to reach any affected site in the brain via ventricles and cerebrospinal fluid, as described by Schulz and Engelhardt [16] in an EAE model.

TrkA Expression in Neurons and CD45⁺ Cells

It has been demonstrated that AP neurons are able to express TrkA during adulthood [42]. Thus, this receptor is a marker of AP neurons while CD45 identifies immune cells; using these markers it is possible to analyze the distribution of both cell types in this structure. The close proximity between neurons and leukocytes found in this

work (fig. 5) could be related to the function of TrkA and their ligands such as nerve growth factor (NGF) during neuroinflammation. NGF released by leukocytes infiltrating the CNS has been suggested as a cross talk between immune cells and neurons through the TrkA receptor in a mouse model of EAE [43]. NGF can also be released by the presence of an immunogen such as LPS [44] or by anxiety-related psychological stress in humans and rodents [45, 46].

On the other hand, contact between neurons and immune cells in the AP has been described during LPS-induced neuroinflammation [14]. This indicates that interaction between immune cells and neurons can be induced by LPS as well as psychological distress. In general, the CNS inflammation induced by chronic psychological distress can develop the same neuroimmunological interactions as immunogenic stimuli do.

TrkA Expression in CD45⁺ Cells

Evidence of TrkA expression in some CD45⁺ cells in the AP (fig. 6) and the increase in this coexpression in the distressed group (fig. 6, 7) could suggest that immune cells are activated in accordance with the following facts. TrkA expression in monocytes is associated with proinflammatory activity and production of reactive oxygen species [47]. TrkA acts as an inflammatory marker in monocytes/macrophages exposed to LPS and NGF [44]. TrkA is also present in lymphocytes of patients with psychiatric disorders, such as schizophrenia, Alzheimer's disease, depression and stress disorders [48]. In addition, TrkA stimulation enhances survival of inflammatory cells, such as monocytes, by protecting them from apoptosis, suggesting the persistence of inflammatory responses in autoimmune disorders, including multiple sclerosis [49]. If leukocytes expressing TrkA⁺ in the AP are activated through this receptor either by the release of NGF from neurons by themselves or via another source, this could prolong leukocyte survival, which may possibly explain prolonged neuroinflammation during stressful sustained episodes resulting in detrimental neural diseases. However, TrkA expression in microglial cells (fig. 6ii) might indicate chemotaxis of microglia via activation of the TrkA receptor, as described in the majority of brain pathologies involving activation and mobilization of microglia [50].

One point to remark is that most of the evidence about leukocyte infiltration into the CNS has been demonstrated by the presence of viral, protozoan and bacterial stimuli [30], or by mechanical injury in animal models and humans [51], i.e. LPS injection [14], HIV dementia [52]

and autoimmune diseases of the CNS [16]. Recently, it has also been demonstrated that inflammatory LPS stimulation as well as psychological stress can trigger the activation of inflammatory transcription factors in different cell types of the brain [53]. Our study is one of few works investigating the recruitment of circulating leukocytes to the CNS following psychological distress [8, 40] and the first one to suggest the AP as a possible route of entry for leukocytes, which indicates that the AP plays an important role during immune responses to distress. Considering the above-mentioned pieces of evidence with our results, we can support the theory that there is only a semantic difference between immunological stimuli such as antigens and stressors [53–55].

Finally, this work provides novel knowledge about how chronic psychological distress (a proinflammatory cause) induces microglial activation, leukocyte recruitment, close proximity between neurons and leukocytes and expression of neuronal molecules by the immune system in the AP. We demonstrated that the AP is immuno-

responsive to distress and it is a possible route of entry for circulating immune cells to reach affected structures in the CNS. These findings suggest multiple roles for immune cells in the AP after chronic psychological distress exposure, such as an increased inflammatory response causing detrimental changes or the healing of damaged tissue. Possible effects on autonomic functions induced by the AP in relation to distress-induced neuroinflammation may be a future topic of study.

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Disclosure Statement

Nothing to report.

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