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Atopic dermatitis induces anxiety- and depressive-like behaviors with concomitant neuronal adaptations in brain reward circuits in mice

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ABSTRACT

Clinically, it has been reported that atopic dermatitis (AD) has been linked with negative emotional problems such as depression and anxiety, thereby reducing the quality of life, but little is known about the molecular mechanism that underlies AD-associated emotional impairments. We sought to determine whether AD could induce anxiety- and depressive-like symptoms in mice and to identify pertinent signaling changes in brain reward circuitry. AD-like lesions were induced by the repeated intradermal application of MC903 into the cheek of the mouse. We assessed dermatitis severity with scratching behavior, histopathological changes, anxiety- and depressive-like behaviors using the elevated plus maze, open field and tail suspension tests, and serum corticosterone levels. In the nucleus accumbens (NAc), dorsal striatum (DS) and ventral tegmental area (VTA), protein levels of dopamine- and plasticity-related signaling molecules were determined by Western immunoblotting assay. Intradermal administration of MC903 into mouse cheek provoked a strong hind limb scratching behavior as well as the robust skin inflammation with epidermal thickening. MC903-treated mice also displayed markedly increased anxiety- and depressive-like behaviors, along with elevated serum corticosterone levels. Under these conditions, enhanced cAMP response element binding protein (CREB) and dopamine and cAMP-regulated phosphoprotein, 32 kDa (DARPP32) phosphorylation, significantly higher brain-derived neurotrophic factor (BDNF) and ΔFosB, but reduced tyrosine hydroxylase (TH) and dopamine D1 receptor (D1R) protein expression were found in the NAc, DS and VTA. Striatal BDNF, phospho-DARPP32 and phospho-CREB levels were significantly associated with the levels of depressive-like behavior in these mice. Taken together, these findings demonstrate that AD-like skin lesion elicits anxiety- and depressive-like phenotypes that are associated with neuroplasticity-related changes in reward circuitry, providing a better understanding of AD-associated emotional impairments.

1. Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disorder with a wide-ranging impact on the quality of life of the patients and their families (de Bruin Weller et al., 2013). Its prevalence has increased over the years and now affects up to 30% of children and up to 17% of adults worldwide (Sacotte and Silverberg, 2018). While AD begins usually in early childhood and often persists into adulthood, it may also start in adults (Margolis et al., 2014).

The main symptoms of AD are dry skin, lichenification, erythematous scaling papules, eczematous inflammation, and persistent intense itching. These clinical symptoms of AD, particularly the unrelenting itch and the negative feeling of the altered physical appearances caused by the eczematous skin lesions impose a psychological burden on AD patients and their families (Buske-Kirschbaum et al., 2001). In fact, increasing evidence shows that both adolescents and adults suffering from AD frequently exhibit a number of psychological distress such as anxiety and depression (Chamlin, 2006; Cheng et al.,

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2015; Hashizume et al., 2005; Linnet and Jemec, 2001). Even mild AD may be accompanied by these emotional states (Kim et al., 2015). Hence, it has become increasingly recognized that the inclusion of interventions for associated psychologic factors may be considered for more effective management of AD (Jafferany, 2007). Even so, despite the increasing importance of the relationship between AD and psychological distress, such as anxiety and depression, little to nothing is known about how the development of AD heightens the risk for anxiety and depression.

Itch, the most prominent complaint in AD, leads to scratching. Despite providing temporary relief of itch at first, ongoing scratching can lead to an increase in the intensity of itch as well as worsening of the skin condition (Ishiuji et al., 2008; Yosipovitch and Papoiu, 2008). Patients with AD are often unable to stop scratching despite negative consequences. This appears to be due to the rewarding properties of scratching. Besides the relief of itch, scratching simultaneously evokes a pleasurable feeling, particularly in chronic itch patients (Mochizuki et al., 2015). In addition, neuroimaging studies have addressed that this pleasurable experience is related to the brain's reward system implicated in the control of reward and motivated behaviors (Mochizuki et al., 2014; Papoiu et al., 2013).

Central to the brain's reward system is the mesolimbic dopamine (DA) system, arising from the ventral tegmental area (VTA) and substantia nigra of the midbrain and projecting to target limbic regions including the nucleus accumbens (NAc) and dorsal striatum (DS). It is recently recognized that, apart from regulating reward and motivated behaviors, mesolimbic DA reward circuit also contributes importantly to mediating negative emotional states such as anxiety, depression, and stress (Nestler and Carlezon Jr, 2006). Recent neuroimaging results show that depressive symptoms are related to reduced activation of the brain's reward circuitry, specifically the NAc, in depressed patients (Satterthwaite et al., 2015). An animal study has also shown that social defeat stress-induced behavioral changes (e.g. depression) are associated with alterations of the mesolimbic dopamine system in animals (Berton et al., 2006). In addition, deep brain stimulation to reward circuitry alleviates anhedonia, anxiety, and depression in patients with treatment-resistant depression (Schlaepfer et al., 2008).

Although there are sufficient reports that AD has been associated with anxiety and depression, how AD modulates negative mood states is relatively unexplored. Given that mesolimbic DA reward system is implicated in both rewarding properties of scratching and negative emotional states, we hypothesized that AD might promote anxiety- and depressive-like behavior that is associated with neural adaptations in brain reward circuitry. Here, to test this, we studied the influence of AD on the development of anxiety- and depressive-like behaviors, and furthermore, investigated whether there are any changes in DA signaling in the striatum and VTA.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (7 weeks old) from Samtaco (Osan, Korea) were housed in cages of 3–5 under standard housing conditions on a 12-h light: dark cycle with free access to food and water at 24 °C. All procedures were conducted in accordance with the Kyung Hee University guidelines for the care and use of laboratory animals and were approved by Kyung Hee University Animal Care Committee for Animal Welfare (IACUC-2017-024-1).

2.2. Induction of AD-like skin lesions using MC903

During brief anesthesia with isoflurane, the right cheek was shaved at least two days before experiments began. For inducing AD-like lesion, 20 μl ethanol alone or containing 1.65 μg MC903 (Sigma-Aldrich, St Louis, Mo) was injected intradermally into the cheek once a day for 7 days (Morita et al., 2015). Lesion severity, scratching behavior, and anxiety- and depressive-like behaviors were observed until 11 days after the first injection (4 days after the last injection). The experimental schedule has shown in Fig. S1.

2.3. Clinical skin score

The severity of AD-like skin lesions was scored following the criteria described previously (Morita et al., 2015). Lesion severity scores were assessed for redness (erythema), dryness (xerosis), and scabbing (excoriation) based each on a 0–3 scale (0, none; 1, mild; 2, moderate; 3, severe) and were summed up. Lesion scoring was performed while blind to treatment.

2.4. Scratching behavior

Scratching behavior was observed on days 3, 5, 7 (ongoing treatment) and days 9 and 11 (post-treatment; Fig. S1). On the day of an experiment, mice were habituated to transparent plastic cylinders (11 cm in diameter; 1 mouse/cylinder) for at least 30 min prior to the beginning of every experiment. Then, immediately after the injection of MC903, mice were put back into the same cylinder and recorded for 30 min to assess scratching. The time spent scratching the injected site was quantified over a 30 min period. One bout of scratching was defined as an episode in which a mouse lifted its hind paw and scratched continuously for any length of time until the paw was returned to the floor (Shimada and LaMotte, 2008). The behavior was counted under blind conditions.

2.5. Histological evaluation

The formalin-fixed skin samples were embedded in paraffin, cut into 5 μm-thick sections, and stained with hematoxylin-eosin for histological examination of the skin. The degree of epidermal hyperplasia was assessed as epidermal thickness measured with ImageJ software.

2.6. Elevated plus maze test

After 3 days of the last MC903 injection (d10), mice were subjected to elevated plus maze (EPM) test. A plus maze apparatus consisted of two open arms (16 × 5 cm) and two enclosed arms (16 × 5 cm with 12 cm high walls) that extend from a central platform (5 × 5 cm), was placed 40 cm above the floor. Each mouse was placed in the center of the EPM facing an open arm that faced away from the experimenter and allowed to freely explore the maze for 5 min in dim lighting. Movement was recorded by an overhead video camera. Time spent and distance traveled in each of the open and closed arms were analyzed with SMART v3.0 (Panlab, Barcelona, Spain) and the number of entries into each of the open and closed arms was counted when the mouse placed all four paws in each arm by a well-trained observer who was blind to the treatment. Percent open arm entries and percent open arm time were used as indices of anxiety-like behavior. Percent open arm entries were calculated as the number of open arm entries divided by total entries (open arm entries plus closed arm entries) and percent open arm time as the amount of time spent in open arms divided by the total amount of time spent in both open and closed arms. Distance traveled (cm) was used as the measure of activity. An anxiety index ranging from 0 (low anxiety) to 1 (high anxiety) was also calculated based on the following formula (Oh et al., 2018): Anxiety Index = 1 − [(time spent in open arm/test duration + number of entries to the open arms/total number of entries)/2].

2.7. Open field test

The open field test (OFT) was carried out 1 day after the EPM. At the beginning of a session, each mouse was placed in the center of a white

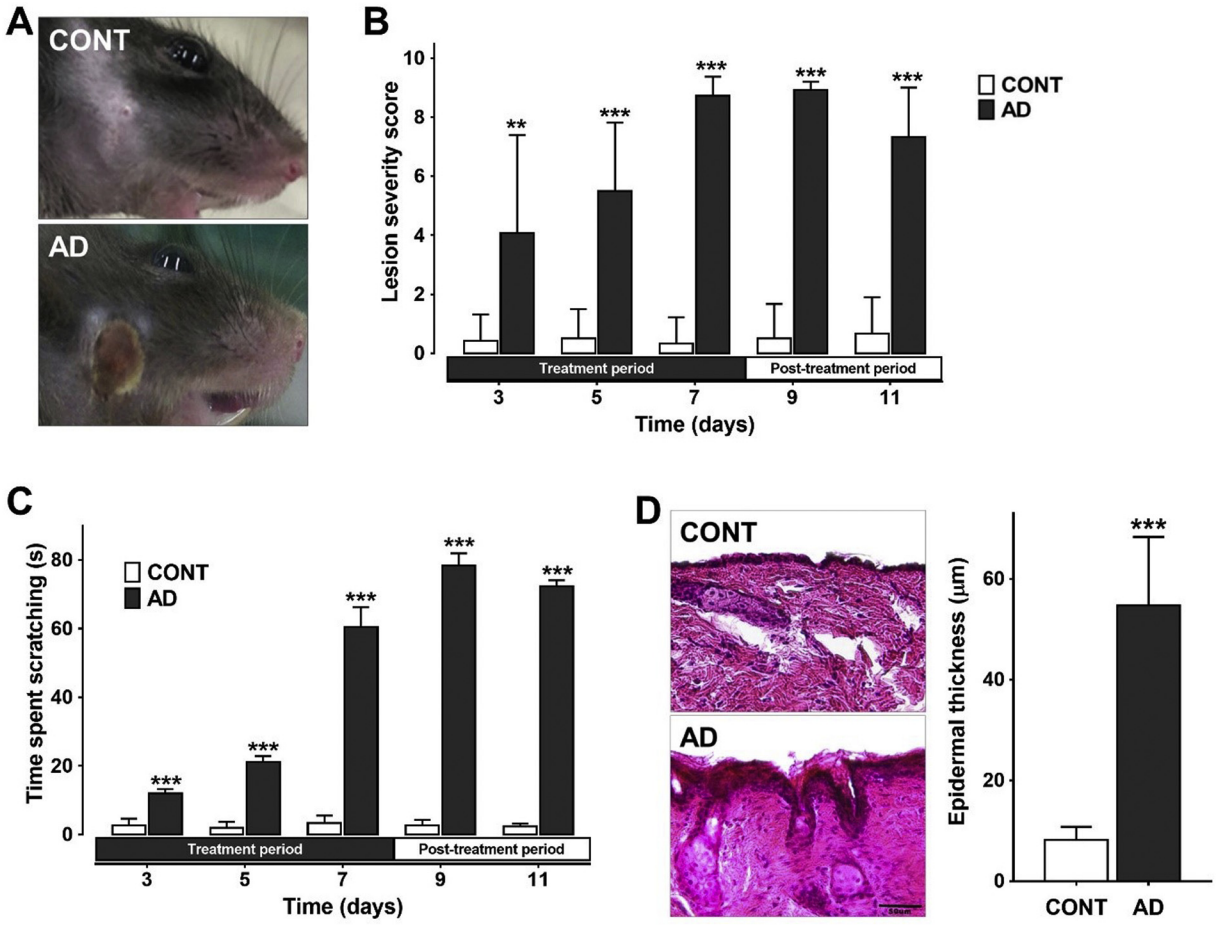

Fig. 1. Development of the AD-like symptoms in mice treated with MC903. (A) Representative appearance of the AD-like skin lesions in mice 4 days after MC903 treatment for 7 days. (B) AD-like lesion severity scores in mice treated with MC903 or vehicle. (C) Scratching behavior during and after treatment with MC903 or vehicle. (D) Hematoxylin and eosin–stained sections of cheek skin from mice 4 days after MC903 treatment for 7 days (Scale bar, 50 μm). (E) Measurements of the epidermal thickness of mice treated with MC903 or vehicle. All data are presented as mean ± SD, $n = 6$ mice/group. $^{**}p < .01$ and $^{***}p < .001$ vs. the CONT group. CONT, vehicle-treated mice; AD, MC903-treated mice.

plastic box measuring 40 × 40 × 27 cm and allowed to freely explore the field for 5 min. Movement was recorded by an overhead camera and analyzed with SMART v3.0 (Panlab, Barcelona, Spain). Measurement included time spent and distance traveled in the center zone.

2.8. Tail suspension test

The tail suspension test (TST) was conducted 30 min after the OFT. For the TST, mice were suspended by the tail taped on a horizontal bar (50 cm above the surface of a table covered with soft padding material). The whole 6-min testing session was video-recorded, and the immobility time was scored during the total time of the test session.

2.9. Serum corticosterone levels

Under isoflurane anesthesia on day 11, blood samples were collected through the cardiac puncture, followed by cervical dislocation to ensure death. Serum was obtained after allowing blood to clot for 30 min at room temperature before centrifugation for 15 min at 1000 ×*g*. Basal corticosterone levels were assessed as an indicator of stress. Serum corticosterone levels were measured by corticosterone ELISA kit (Abcam, Cambridge, MA, USA) according to the manufacturer's instructions.

2.10. Immunoblot assay

Levels of ΔFosB, cyclic AMP-response element binding protein (CREB), phospho-CREB (pCREB), brain-derived neurotrophic factor (BDNF), dopamine D1 receptor (D1R), dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP32), phospho-Thr34 DARPP32 (pDARPP32), tyrosine hydroxylase (TH), and β-actin were analyzed by immunoblot assay. Briefly, mouse brains were quickly removed immediately after the blood collection and sliced into 1-mm coronal sections using a mouse brain matrix. The NAc, DS, and VTA were microdissected bilaterally using brain tissue punches (Stoelting, Chicago, IL, USA). Brain tissue lysates from the NAc, DS, and VTA were prepared in lysis buffer (CyQUANT; Invitrogen, Eugene, OR, USA) with protease inhibitors (Roche Applied Science, Mannheim, Germany) and PhosStop phosphatase inhibitors (Roche Applied Science). Protein concentrations were determined using a BCA Protein Assay kit (Thermo Scientific, Rockford, IL, USA). Equal amounts of total protein samples were electrophoresed on 10% PAGE gels and transferred to PVDF membrane. Following incubation with antibodies against ΔFosB (1:500; Cell Signaling Technology Inc., Danvers, MA, USA), pCREB (1:500; Cell Signaling Technology), total CREB (1:500; Cell Signaling Technology), BDNF (1:300; Santa Cruz Biotechnology, Santacruz, CA, USA), DA D1A receptor (1:250; Santa Cruz Biotechnology), pDARPP32 (1:100; Cell Signaling Technology), total DARPP32 (1:100; Cell Signaling Technology), TH (1:1000; Santa Cruz Biotechnology) or β-actin (Sigma-Aldrich, St. Louis, MO, USA), blots were visualized with using the Super

Signal West Pico Chemiluminescent Substrate (Thermo Scientific). Band intensities of scanned films quantified by densitometry using ImageJ software. Phosphoproteins were normalized to their respective total proteins and non-phosphoproteins were normalized to β-actin loading control. Changes in protein levels are presented as a percentage relative to the controls, which were denoted as 100%.

2.11. Statistical analysis

All data are presented as means ± SD. Unpaired two-tailed *t*-test was used to compare the means of two groups. Pearson correlation and linear regression analyses were used to assess the relationships between TST immobility and protein levels of reward-related signaling molecules. All statistical analyses were performed in GraphPad Prism v7.0 (GraphPad software). Differences were considered statistically significant if $p < .05$.

3. Results

3.1. Vitamin D3 analog MC903 induces experimental AD-like symptoms

As a vitamin D analog, MC903-induced mouse model of AD shows AD-like phenotypes that are similar to those in humans with this disorder - the development of eczematous lesions, skin hyperplasia and the intense itch-evoked scratching (Li et al., 2017, Morita, McClain, 2015), we chose this model to verify that AD is associated with depression or anxiety. We initially examined whether mice treated with MC903 develop eczematous-like lesions. Mice were treated daily with MC903 by intradermal application into the cheek for 7 consecutive days to induce AD-like symptoms and monitored itch behaviors as well as skin lesion severity for a total of 11 days. Ethanol was used as vehicle control. Intradermal application of MC903 induced changes in skin morphology and inflammation (Fig. 1A). The cheek of MC903-treated mice became red, dry and scaly and worsened over time (Fig. 1B). MC903-treated mice also displayed scratching behaviors that began on the third day of MC903 treatment and increased in intensity, even after the 7-day treatment period (Fig. 1C). These itch behaviors persisted 4 days after the last skin treatment (d11), establishing this paradigm as a model of chronic rather than acute itch. Histological analysis showed that on the application of MC903, mice developed severe skin inflammation with heavy infiltrations in the dermis and thickening of the epidermis (Fig. 1D and F). Ethanol application did not cause any change in cheek skin.

3.2. MC903-induced AD elicits anxiety- and depressive-like behavior

Next, to determine if MC903-induced AD would result in increased anxiety and depression, we first tested mice for anxiety-like behavior using the EPM. MC903-induced AD mice entered significantly less into and spent significantly less time in the open arms compared to the controls (Fig. 2A and B), indicative of a higher state of anxiety. Consistent with these findings, the anxiety index that integrates the EPM behavioral measurement was significantly higher for MC903-treated mice compared to those of vehicle-treated controls (Fig. 2C). Total distance traveled in the EPM and a total number of entries into open and closed arms did not differ between both groups (Fig. S2). We confirmed these findings using the OFT. Consistent with the EPM result, the time spent and distance traveled in the center of the OF were significantly lower for MC903-treated mice than control mice (Fig. 2D and E). We next assessed depressive-like behavior using the TST, a measure of behavioral despair. MC903-treated mice showed a pronounced increase in behavioral despair when compared to the untreated control group, as indicated by a significantly increased time spent immobile in the TST (Fig. 2F).

3.3. Altered serum corticosterone levels after MC903 treatment

Elevated levels of glucocorticoids have been implicated in psychiatric disorders including anxiety and depression (Wolkowitz et al., 2009). To determine if MC903-induced AD increases basal corticosterone levels, we measured serum corticosterone levels in mice 4 days after the administration of MC903 for 7 days. MC903-induced AD mice showed significantly higher corticosterone levels compared to ethanol-treated control mice (Fig. 3).

3.4. MC903-induced AD alters neuronal plasticity and DA-related molecules in the mesolimbic system

To determine if MC903 treatment and the resulting AD give rise to adaptations in dopaminergic brain regions (the NAc, DS, and VTA), we analyzed the expression of proteins relevant to neuronal plasticity and DA function by western blot. We first focused on the NAc, the reward center of the brain. In the NAc, there was a modest, yet statistically significant, increase in ΔFosB protein levels (1.2-fold, $p < .05$; Fig. 4A). When compared with to the control mice, MC903 also led to significantly increase the activation of CREB as indicated by the ratio of levels of p-CREB/total CREB, and increased expression of BDNF (1.5-fold, $p < .001$ and 1.6-fold, $p < .001$, respectively; Fig. 4A). Expression of DA D1R protein was significantly decreased by about 50% after MC903 expose as compared with control mice ($p < .05$; Fig. 4B). However, we observed that MC903-induced AD-like lesions led to significantly higher levels of p-DARPP32 (Thr34) protein in the NAc as compared with controls (1.5-fold, $p < .001$; Fig. 4B). TH protein levels were slightly decreased by MC903, but this was not statistically significant (0.75-fold, $p = .062$; Fig. 4B). Similar to the results in the NAc, MC903 treatment and the resulting AD significantly increased the protein levels of ΔFosB (1.4-fold, $p < .01$), pCREB (3.4-fold, $p < .001$), and BDNF (2.0-fold, $p < .001$) in the DS (Fig. 4C). Further, D1R (0.34-fold, $p < .001$) and TH (0.58-fold, $p < .01$) were decreased, whereas phosphorylation of DARPP32 (1.52-fold, $p < .001$) was increased after MC903 treatment (Fig. 4D). Similar results were also observed in the VTA. ΔFosB (1.23-fold, $p < .01$), pCREB (1.38-fold, $p < .01$), and BDNF (1.53-fold, $p < .05$) levels were all higher in the MC903 treatment group than those in the CONT group (Fig. 4E). Both D1R (0.52-fold, $p < .01$) and TH (0.22-fold, $p < .05$) were significantly decreased after MC903 treatment as compared with the control group (Fig. 4F). Phosphorylation levels of DARPP32 were unchanged in the VTA of MC903-treated mice (Fig. 4F).

3.5. Depressive behavior associated with MC903-induced AD is positively correlated with striatal BDNF and pCREB

To examine whether depressive-like behavior correlated with reward-related signaling molecules, we further analyzed the relationship between immobility in the TST and the expression levels of neuronal plasticity- and DA-related proteins. Time spent immobile in the TST was positively correlated with pCREB ($R^2 = 0.8785$, $p < .001$; Fig. 5D), BDNF ($R^2 = 0.8176$, $p < .05$; Fig. 5G) and pDARPP32 ($R^2 = 0.6827$, $p < .05$; Fig. 5M), and negatively related to D1R ($R^2 = 0.5425$, $p < .05$; Fig. 5J) in the NAc. No correlations were found between immobility and ΔFosB ($R^2 = 0.4546$, $p = .065$; Fig. 5A) or TH ($R^2 = 0.3876$, $p = .099$; Fig. 5P). In the DS, ΔFosB ($R^2 = 0.6512$, $p < .05$; Fig. 5B), pCREB ($R^2 = 0.8335$, $p < .01$; Fig. 5E), BDNF ($R^2 = 0.7467$, $p < .01$; Fig. 5H), and pDARPP32 ($R^2 = 0.8723$, $p < .001$; Fig. 5N) were positively correlated with levels of immobility, while D1R ($R^2 = 0.9352$, $p < .001$; Fig. 5K) and TH ($R^2 = 0.876$, $p < .001$; Fig. 5Q) were negatively correlated with it. We also observed strong positive correlations of immobility with ΔFosB ($R^2 = 0.7206$, $p < .01$; Fig. 5C) and BDNF ($R^2 = 0.5127$, $p < .05$; Fig. 5I) in the VTA, and strong negative correlations of it with D1R ($R^2 = 0.8323$, $p < .01$; Fig. 5L) and TH ($R^2 = 0.8951$, $p < .01$;

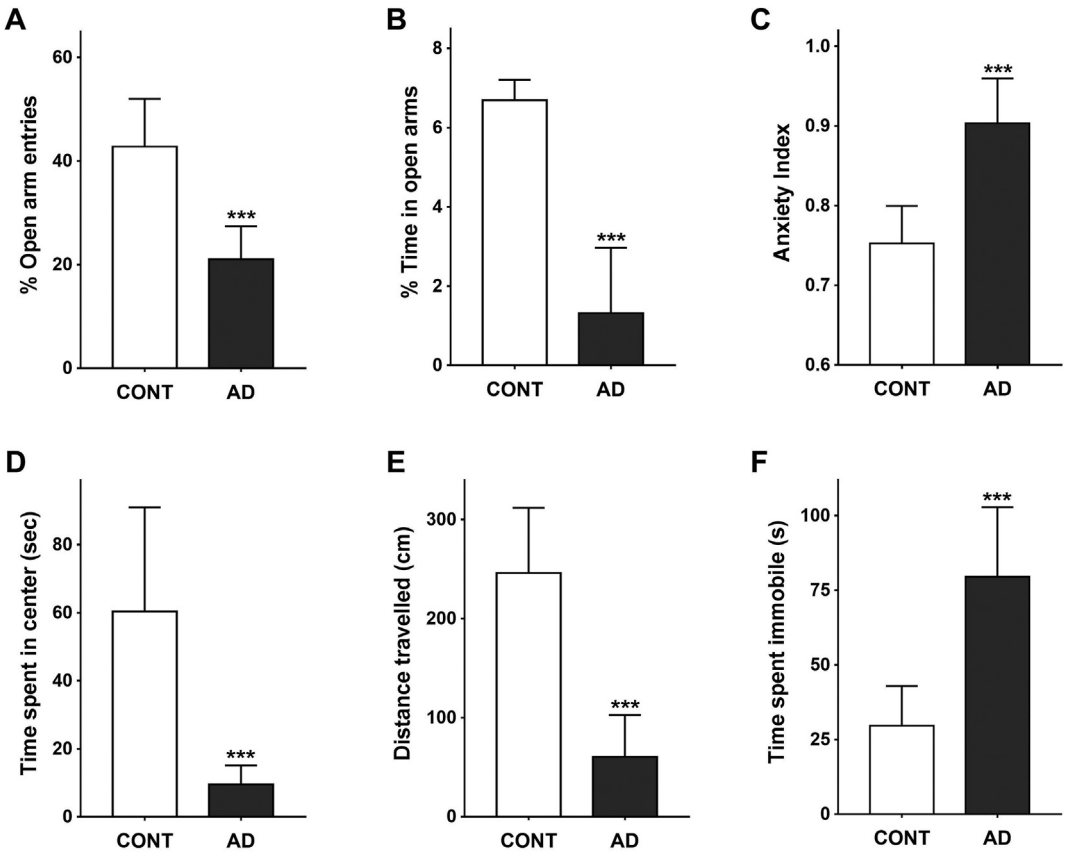

Fig. 2. Alterations in anxiety- and depressive-like behaviors after the development of MC903-induced AD-like lesions. MC903-treated AD mice were tested for anxiety-like behavior in the elevated plus maze (EPM; A-C) and the open field test (OFT; D and E) 3 days after the last MC903 treatment and for depressive-like behavior in the tail suspension test (TST; F) 4 days after the last MC903 treatment. Percentage of open arm entries (A), percentage of time spent in the open arms (B) and anxiety index (C) in the EPM test, time spent in the center (D) and distance traveled in center (E) in the OFT, and immobility time in the TST (F) in mice treated with MC903 or vehicle. All data are presented as mean ± SD, n = 6 mice/group. ***$p < .001$ vs. the CONT group. CONT, vehicle-treated mice; AD, MC903-treated mice.

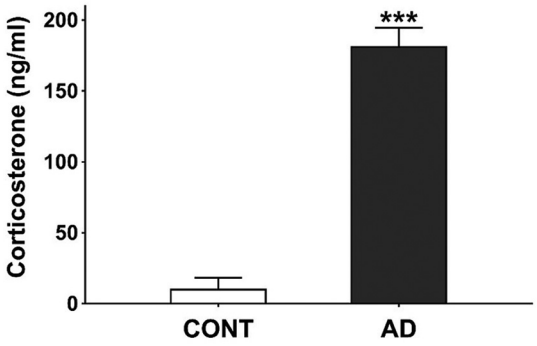

Fig. 3. Changes in basal corticosterone levels Serum corticosterone levels after the development of MC903-induced AD-like lesions. Serum corticosterone levels were measured in CONT and AD mice 4 days after the administration of MC903 for 7 days. All data are presented as mean ± SD, n = 6 mice/group. ***$p < .001$ vs. the CONT group. CONT, vehicle-treated mice; AD, MC903-treated mice.

Fig. 5R) in the VTA. Poor or no correlations were found between immobility levels and pCREB ($R^2 = 0.4831$, $p = .056$; Fig. 5F) or pDARPP32 ($R^2 = 0.03731$, $p = .6467$; Fig. 5O) in the VTA.

4. Discussion

A growing body of data suggests that AD is associated with an increased risk of developing anxiety and depression (Cheng et al., 2015, Dommasch et al., 2015), but less is known about the possible mechanism that underlies the link between AD and vulnerability to anxiety and depression. In this study, we found that the development of AD-like skin lesions by repeated topical application with MC903 promoted anxiety- and depressive-like behavior, led to a heightened state of stress, and elicited neuroplastic changes in the striatum and VTA. Our study demonstrates for the first time that AD-like skin lesion that is concomitant with itch has pro-anxiety and pro-depressive effects that are associated with increases in pDARPP32, pCREB and BDNF in the striatum, signals that are implicated in reward and neuroplasticity. These findings propose a possibility whereby AD increases the levels of pDARPP32, pCREB and BDNF in the striatum that contribute to negative emotional states such as anxiety and depression.

As mentioned above, negative emotional states such as anxiety and depression are commonly observed in patients with AD. In our experiments, AD mice induced with MC903 exhibited anxiety- and depressive-like behaviors characterized by reduced time spent in open areas (open arms in the EPM and the center of the open field) and increased immobility in the TST, respectively. The observed changes in behaviors were unlikely due to general locomotor impairment, as evidenced by no difference in total distance traveled in the EPM and the total number of entries into open and closed arms between both groups. Furthermore, in agreement with previous studies reporting high stress levels in AD patients (Park and Kim, 2016) and increased corticosterone levels in AD mice (Park et al., 2018), our results indicated that AD mice with anxiety- and depressive-like phenotypes are in a heightened state of stress, as evidenced by elevated serum corticosterone levels. Thus, our findings might show that AD mice demonstrate an inclination

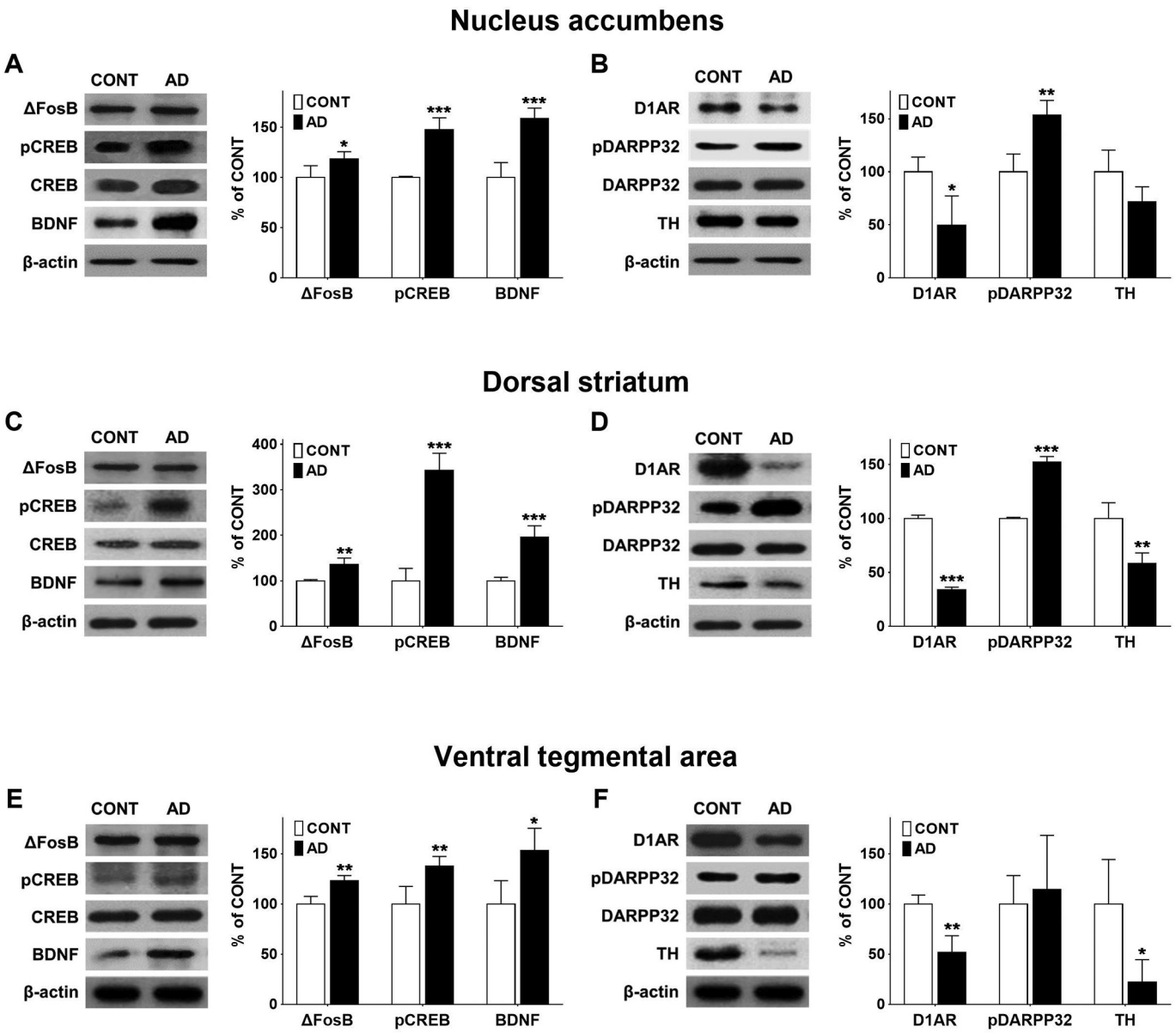

Fig. 4. Changes in reward signaling pathway in the brain reward regions after the development of MC903-induced AD-like skin lesions. Representative western blots of plasticity-related (A, C, and E; ΔFosB, pCREB and BDNF) and DA-related proteins (B, D, and F; D1AR, pDARPP32 and TH) in the nucleus accumbens (A and B), dorsal striatum (C and D), and ventral tegmental area (E and F) of CONT and AD mice 4 days after the administration of MC903 for 7 days. The *right panel* of each blot shows the average percentage change in densitometry, normalized to β-actin (for ΔFosB, BDNF, D1AR and TH), total CREB (for pCREB) or total DARPP32 (for pDARPP32) and relative to CONT. All data are presented as mean ± SD, $n = 4$ mice/group. $^{*}p < .05$, $^{**}p < .01$ and $^{***}p < .001$ vs. the CONT group. CONT, vehicle-treated mice; AD, MC903-treated mice.

similar to that of patients with AD for emotional states such as stress, anxiety, and depression, making this a useful behavioral model in the study of psychobiological aspects of AD.

So far, very little to nothing is known about the mechanisms by which AD might evoke negative emotional states. Emerging findings indicate that scratching an itch evokes a rewarding and pleasurable sensation as well as itch relief (Bin Saif et al., 2012) and that brain's reward circuits mediate scratching-related itch relief (Papoiu et al., 2013). In parallel, there is considerable evidence that the altered function of midbrain-striatal DA reward circuits is implicated in the pathogenesis of depression and anxiety often seen in addiction to abused drugs like alcohol or to natural rewards like food, particularly those rich in fat and sugars (Luthi and Luscher, 2014; Sharma and Fulton, 2013). These observations have led to the hypothesis that neuroplastic adaptations in brain reward circuits may underlie the association of AD with anxiety and depression.

Since the dopaminergic cells projecting from the VTA to the striatum are thought to be key players in the brain reward system playing a significant role in hedonic and emotional processes implicated in the psychiatric disorders (Nielsen et al., 2016), we centered our studies in the striatum (NAc and DS) and VTA. Neural adaptations require molecular changes such as an increase in transcriptional and neurotrophic signals (Nair et al., 2014, Nestler and Carlezon Jr, 2006). We first examined levels of D1 receptor and downstream effectors as markers indicative of a dysregulation in DA signaling in the NAc, DS, and VTA. Accompanying increased anxiety and depression in AD mice were reduced D1R and TH levels in all the NAc, DS, and VTA, suggesting reduced DA tone in the brain reward circuits and altered feedback from the NAc to the VTA. These observations are consistent with the previous reports of reduced dopaminergic activity in both

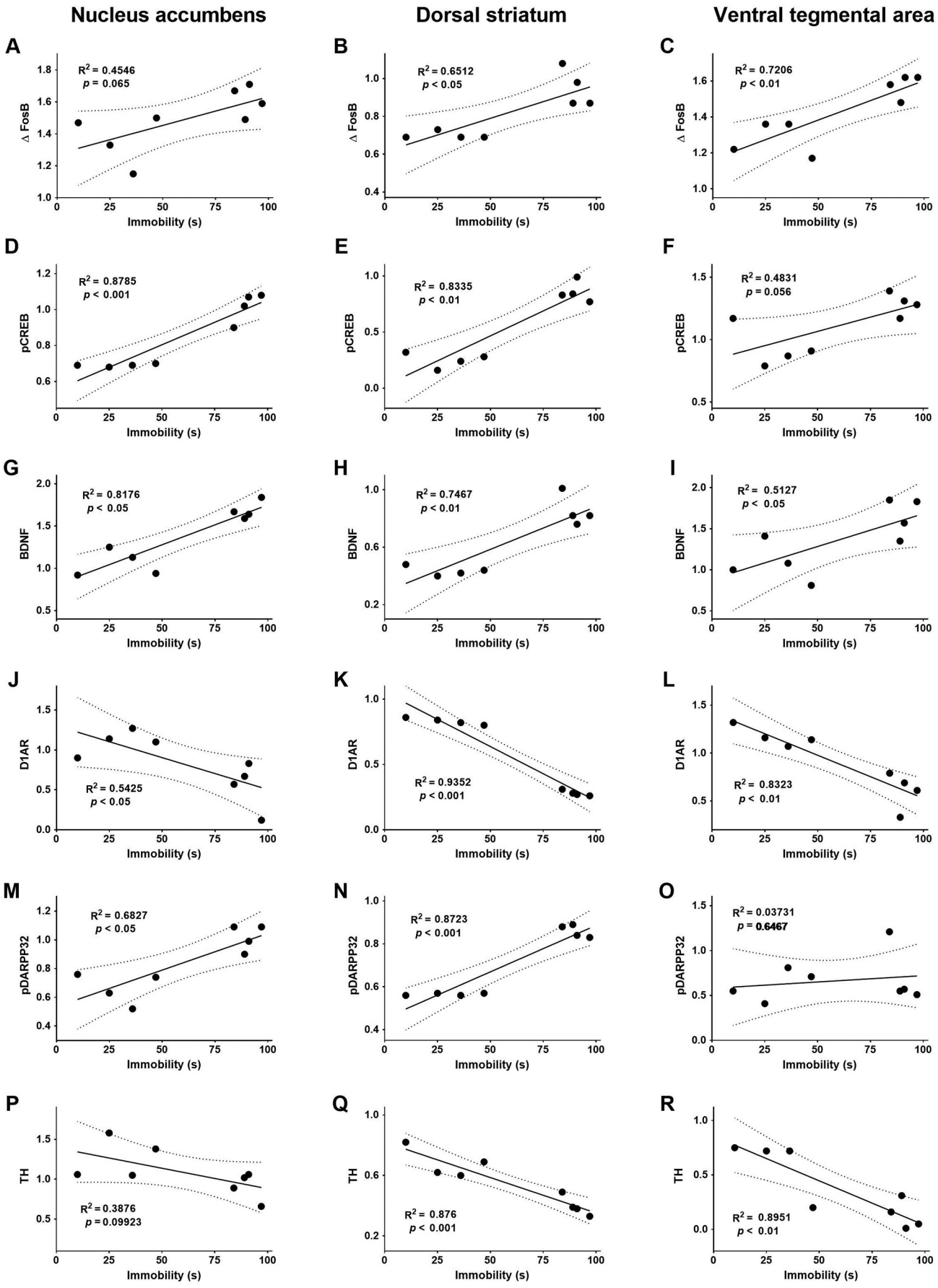

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Fig. 5. Correlations of depressive-like behavior with reward-related signaling molecules in the brain reward regions after the development of MC903-induced AD-like skin lesions. Pearson's correlation and linear regression analysis were performed to test potential associations of the immobility time in the tail suspension test with protein levels of ΔFosB (A-C), pCREB (D-F), BDNF (G-I), D1AR (J-L), pDARPP32 (M-O) and TH (P-R) in the nucleus accumbens, dorsal striatum, and ventral tegmental area of CONT and AD mice. Best-fit linear regression lines are shown together with R^2 and exact p values in each graph. Dotted lines represent 95% confidence interval. For all graphs, each data point corresponds to the values from one mouse. Values of $p < .05$ were considered statistically significant. CONT, vehicle-treated mice; AD, MC903-treated mice.

depressed individuals and animals (Belujon and Grace, 2017). Interestingly, phosphorylation levels of DARPP32 at Thr34 were significantly increased in these brain regions of AD mice, which is inconsistent with several reports that D1 receptor stimulation results in an increased phosphorylation of DARPP32 at Thr34 in response to PKA activation and as a result to inhibit protein phosphatase I (PP1), leading to potentiation of dopaminergic signaling (Walaas et al., 2011). This discrepancy is probably explained by the regulation of DARPP32 phosphorylation by the signaling of other neurotransmitters besides DA (Walaas et al., 2011). Serotonin, for example, has an important role in regulating DARPP-32 phosphorylation at Thr34 primarily via activation of serotonin 5-HT4 and 5-HT6 receptors and associated cAMP-PKA pathway (Svenningsson et al., 2004). Glutamate and nitric oxide also stimulate the phosphorylation of DARPP32 at Thr34 by activating glutamate mGluR5 receptor/adenosine A2A receptor/cAMP/PKA and soluble guanylyl cyclase/PKG signaling, respectively (Svenningsson et al., 2004).

In addition, in order to evaluate the effects of AD on neural plasticity, we measured the expression changes in ΔFosB, pCREB, and BDNF based on their roles in neuronal plasticity. We found that there was a slight, significant increase in the levels of ΔFosB in the NAc, DS, and VTA of AD mice. ΔFosB is a unique transcription factor that plays an essential role in long-term adaptive changes underlying neural plasticity and reward learning (McClung et al., 2004). Interestingly, the accumulation of ΔFosB in the NAc and DS results in increased expression of cyclin-dependent kinase 5 (Cdk5) levels, which in turn phosphorylates DARPP32 at Thr75. Increased Cdk5-dependent phosphorylation of DARPP32 at Thr 75 then attenuates D1 dopamine receptor signaling, contributing to adaptive changes (Bibb et al., 2001). Accordingly, in our studies, it appears that AD-like lesions possibly induce a slight, significant increase in the levels of ΔFosB in brain reward circuits that in turn alters the phosphorylation levels of pDARPP32 at Thr75 resulting in decreased DA D1 receptor and dysregulation of DA signaling. Another transcription factor, CREB has been also implicated in the pathophysiology of depression (Berton et al., 2006). CREB is activated by phosphorylation in response to exposure to various stimuli including psychostimulant drugs, and its activation in the NAc has been linked to a variety of emotional responses, as evidenced by findings that elevations of CREB activity in the NAc lead to anhedonia and depressive-like behavior while reduced CREB activity in this region promotes reward (Manning et al., 2017; Muschamp et al., 2011; Pliakas et al., 2001). In our studies, we showed increases in the activation of CREB after the development of AD-like lesions in the NAc, DS, and VTA and a significant positive association of the levels of pCREB with measures of depressive-like behavior in the NAc and DS. Accordingly, the ability of the AD-like lesions to increase CREB phosphorylation is likely related to elevated risk for depressive-like behavior. A key target of CREB signaling, BDNF is a key positive regulator of neural plasticity. Our present study shows that AD-like lesions significantly increased the levels of BDNF in the brain reward regions. We have also observed that BDNF levels in these areas showed a significant positive relationship with the immobility in TST, indicating increases in BDNF may be responsible for the observed depressive-like behavior after the development of AD. Previously it has been reported that BDNF regulates hedonic behavior by acting on the mesolimbic DA system (Cordeira et al., 2010). In addition, while increased BDNF levels in the NAc or VTA produces a depressive-like phenotype, a selective loss of BDNF expression within the VTA oppose the development of depressive-like behaviors in defeat-stressed mice (Berton et al., 2006). Thus, our results of elevations in BDNF levels in reward-related brain areas accompanying increased anxiety and depression in AD mice hold well with these previous findings.

5. Conclusions

Taken together, this study indicates that the development of AD-like skin lesions leads to anxiety- and depressive-like behaviors, which may be associated with neuronal adaptation in reward-related brain regions, in particular striatum. This is, to our knowledge, the first evidence in an animal model that experimental AD has anxiogenic and pro-depressant effects, and the first study to investigate neuronal adaptation at a molecular level as a mechanism by which AD promotes negative emotional states. These findings extend results from experiments indicating that the brain's reward circuits mediate scratching-related itch relief and provide insight into the molecular basis that underlies AD-induced emotional impairments. Future studies should, however, seek to investigate whether the development of AD lesions precede the onset of altered emotional behavior and to provide a more direct link between alterations in neural adaptations in brain reward circuits and the development of AD-associated negative emotional states.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forpol.2018.03.009.

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