

## Expression and localization of $\alpha_{2A}$ -adrenergic receptor in the rat post-natal developing cochlea

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### ABSTRACT

Lots of adrenergic receptors (ARs) are widely present across the auditory pathways and are positioned to affect auditory and vestibular functions. However, noradrenergic regulation in the cochlea has not been well characterized. In this study, a rat model of noise-induced hearing loss was developed to investigate the expression of  $\alpha_{2A}$ -adrenergic receptor (AR) after acoustic trauma, then, we investigated the expression of  $\alpha_{2A}$ -AR in the developing rat cochlea using immunofluorescence, qRT-PCR, and Western blotting. We found that the expression of  $\alpha_{2A}$ -AR significantly increased in rats exposed to noise compared with controls. Immunofluorescence analysis demonstrated that  $\alpha_{2A}$ -AR is localized on hair cells (HCs), spiral ganglion neurons (SGNs), and the stria vascularis (SV) in the postnatal developing cochlea from the post-natal day (P) 0 to P28. Furthermore, we observed  $\alpha_{2A}$ -AR mRNA reached a maximum level at P14 and P28 when compared with P0, while no significant differences in  $\alpha_{2A}$ -AR protein levels at the various stages when compared with P0. This study provides direct evidence for the expression of  $\alpha_{2A}$ -AR in HCs, SGNs, and the SV of the cochlea, indicating that norepinephrine might play a vital role in hearing function within the cochlea through  $\alpha_{2A}$ -AR.

**Key words:** adrenergic receptor; hair cells; spiral ganglion neurons; stria vascularis; rat.

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**Conflict of interest:** the authors declare that they have no competing interests, and all authors confirm accuracy.

**Ethics approval:** all animal procedures were conducted in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of the Air Force Medical University (Ethic Code: IACUC-20220192), Xi'an, Shannxi Province, China.

**Availability of data and materials:** All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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## Introduction

The mammalian cochlea consists of hair cells (HCs), spiral ganglion neurons (SGNs) and stria vascularis (SV), which play an important role in auditory function. Morphological studies have confirmed that the sympathetic nerve fibers are distributed in the cochlea,<sup>1-5</sup> but no adrenergic nerve terminals have been found in the organ of Corti or SV.<sup>6</sup> Therefore, the way by which the sympathetic nervous system (SNS) regulates peripheral auditory function is still not clear. The neurotransmitters in postganglionic sympathetic nerve fibers mainly include norepinephrine (NE), epinephrine, acetylcholine (ACh), purine, and polypeptides. Noradrenergic regulation of the central auditory system occurs at several levels, including the cochlear nucleus,<sup>7</sup> the lateral superior olive,<sup>8</sup> medial geniculate nucleus,<sup>9,10</sup> and auditory cortex,<sup>11-14</sup> where NE alters intrinsic and synaptic physiology. NE exerts a modulatory effect on neuronal activities through the activation of  $\alpha_2$ -adrenergic receptors (ARs).<sup>15-20</sup> However, noradrenergic regulation in the cochlea has not been well characterized. Sympathetic neurons influence activity in target organs by releasing NE through ARs to play a role in target organs.<sup>21</sup> There are three types of ARs exist:  $\alpha_1$  ( $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{1D}$ ),  $\alpha_2$  ( $\alpha_{2A}$ ,  $\alpha_{2B}$ ,  $\alpha_{2C}$ ) and  $\beta$  ( $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ) receptors. These ARs belong to the superfamily of G protein-coupled receptors (GPCRs),<sup>22</sup> which have many key findings in the cochlea, such as stem cell fate, planar cell polarity (PCP), hearing loss, and hearing protection.<sup>23,24</sup> The  $\alpha_1$ ,<sup>25</sup>  $\alpha_{1A}$ ,<sup>26</sup>  $\alpha_2$ ,<sup>27-31</sup>  $\beta$ ,<sup>32,33</sup>  $\beta_1$ ,<sup>25,34,35</sup> and  $\beta_2$ ,<sup>25,35-37</sup> ARs are widely expressed across the peripheral auditory system, with varying expression patterns, and are well positioned to influence inner ear function. However, little is known about developmental changes in cochlear ARs expression.

Experiments using stimulation or transection have documented a protective effect in the SNS regarding noise-induced hearing loss (NIHL).<sup>38-42</sup> However, *Dbh*<sup>-/-</sup> mice lacking measurable NE or epinephrine were as susceptible to noise exposure as controls.<sup>43</sup> Cellular localization studies have provided some insights regarding the possible role of  $\alpha_2$ -ARs in inner ear function.<sup>27</sup> We previously reported that NE suppression of the GABA response is regulated by  $\alpha_2$ -ARs, which reduce intracellular cAMP formation through the inhibition of adenylyl cyclase.<sup>30</sup> Although there are multiple pathways by which the SNS modulates hearing function, the mechanisms of ARs in the development of the rat auditory system are unclear. To address these in the present study, firstly, we observed the  $\alpha_{2A}$ -AR expression in a rat model of NIHL to demonstrate that the receptor may play a role in auditory process; after that, we investigated the expression of  $\alpha_{2A}$ -AR in the developing rat cochlea *via* immunohistochemistry, qRT-PCR analysis, and Western blotting.

## Materials and Methods

### Animals

Sprague-Dawley rats were purchased from the Laboratory Animal Center of the Air Force Medical University. All the experiments were approved by the Institutional Animal Care and Use Committee of the Air Force Medical University (Ethical Code: IACUC-20220192), Xi'an, Shanxi Province, China. Post-natal rats were decapitated without anesthesia while adult animals were decapitated under deep anesthesia *via* an intraperitoneal injection of 10% chloral hydrate (250 mg/kg).<sup>44</sup> Rats were housed under standard conditions (temperature 23±2°C, humidity 60±5%, 12 h light/dark cycle).

### Noise exposure

In this study, rats of both sexes (8-10 weeks old, weighing 180-250 g) were exposed to white noise at 115 dB sound pressure level (SPL) for 6 h per day for two consecutive days. The control group was placed in the noise booth but not exposed to noise. Exposure to noise was performed in a double-walled soundproof room where rats had free access to food and water. A Radio Shack Super Tweeter (Tandy Corp, Fort Worth, TX, USA), which was located above the cages, generated a noise (white noise, 115 dB SPL) that was then amplified by a power amplifier (Yamaha, Japan) and delivered to a loudspeaker. Sound levels of the noise exposure were confirmed by a sound level meter (Bruel and Kjaer, Shanghai, China) at multiple locations within the cabinet to ensure uniformity of the sound field and were measured before and after exposure to ensure stability.

### Auditory brainstem responses

After rats were anesthetized, the active electrodes were inserted into subcutaneous tissue at the vertex, the reference electrode was inserted in the mastoid area of the test ear, and the ground electrode was placed at the base of the tail. Tucker Davis Technology (TDT) System-III hardware and SigGenRZ/BioSigRZ software (Tucker-Davis Technologies, Alachua, FL, US) were used to record the response. Alternating click stimuli were used for response detection and delivered using a headphone MF1 1172, which filtered the acoustic range to 100-3000 Hz. The speaker was in this experiment 1 cm away from the outer ear canal of the rat. Stimulus began with 90 dB intensity (21/s) of tone-burst (frequencies of 4, 8, 16, 24 and 32 kHz), with 10 dB decreased once, and then in 5 dB intervals near threshold until no responses were detected. The first wave was used to determine thresholds for each frequency.

### Distortion product otoacoustic emissions (DPOAEs)

The emissions were measured using a TDT WS-8 computer workstation (Tucker-Davis Technologies). DP-grams of DPOAE amplitudes as a function of f1 and f2 frequencies were represented. Frequencies were acquired with an f2/f1 ratio of 1.2. At one day after noise exposure, frequency was incremented from 4 kHz to 32 kHz through 2 kHz and 4 kHz steps. The detection threshold was determined as a DPOAE of 3 dB above the noise floor.

### Immunofluorescence

For immunofluorescence analysis, the cochleae from post-natal day (P)0, P7, P14, P21 and P28 rats were fixed overnight at 4°C in 4% paraformaldehyde in phosphate-buffered saline (PBS). The tissues were decalcified in 10% EDTA in PBS (P0, P7, and P14 for 2 h; P21 and P28 for 3 to 7 days), followed by dehydration in 30% sucrose solution overnight at 4°C. The samples were embedded in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA, USA) at -20°C, and mid-modiolar 10- $\mu$ m-thick cross-sections of the cochleae were obtained using a cryostat microtome (CM1850; Leica, Wetzlar, Germany). The sections were collected on glass slides.

The cochlear sections were incubated for 40 min at 37°C with a blocking solution made of PBS (0.1% Triton X-100 in PBS) with 5% normal donkey serum (NDS; Jackson ImmunoResearch, West Grove, PA, USA). The primary antibody was applied to the cochlea sections overnight at 4°C. For primary antibodies, we used anti- $\alpha_{2A}$ -AR (ab85570, 1:200; Abcam, Cambridge, UK), and anti- $\beta_3$  (TUJ-1; MAB1637, 1:1000; Millipore, Burlington, MA, USA). After rinsing three times in PBS, secondary antibody incubation was performed at room temperature for 2 h. For secondary antibodies, we used two types of donkey anti-rabbit: A-21206 (Alexa 488 conjugated, 1:200; Thermo Fisher Scientific, Waltham, MA, USA) or AP182C (Alexa 594 conjugated, 1:200; Millipore). After

washing in PBS, the sections were labeled with rhodamine-conjugated phalloidin<sup>45</sup> (PHDR1, 1:200; Cytoskeleton, Inc., Denver, CO, USA) for 10 min at room temperature in the dark. Sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI; 1:1000; Solarbio Corporation, Beijing, China) for 10 min at room temperature in the dark and mounted on glass slides with Mowiol 4-88 (81381-50G; Sigma Aldrich, Steinheim am Albuch, Germany) mounting medium. Every experiment also included a negative control where the primary antibody was omitted.

The sections were observed using a fluorescence microscope (Olympus Corp., Tokyo, Japan). The images were captured using Olympus confocal software FV10-ASW 1.7a and adjusted for brightness and contrast.

### mRNA expression levels of $\alpha_{2A}$ -AR

Cochlea were obtained from Sprague-Dawley rat pups of both sexes (P0, P7, P14, P21, and P28). Total RNA was extracted at 4°C using the E.Z.N.A.<sup>®</sup> Total RNA Kit I (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocol. The obtained total RNA was reverse transcribed into complementary DNA (cDNA) using LifeECO TC-96/G/H(b)C according to the manufacturer protocols (Bioer Technology, Hangzhou, China) *via* the RevertAid First Strand cDNA Synthesis Kit (K1622; Thermo Fisher Scientific). Gene-specific mRNA analyses were performed using the standard protocol for TB Green<sup>®</sup> Premix Ex Taq<sup>™</sup> II (TaKaRa Biotechnology, Kusatsu, Japan). The 2<sup>- $\Delta\Delta C_t$</sup>  method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous reference was used to determine the relative levels of gene expression. The primer sequences for  $\alpha_{2A}$ -AR,<sup>46, 47</sup> and GAPDH<sup>47</sup> are listed in Table 1.

### Western blotting

At least six cochlea tissues (P0, P7, P14, P21, P28, and P56-P70) were collected for each Western blot. Total proteins were extracted from the cochlea *via* the Protein Extraction Kit (Solarbio, Beijing, China). Sample protein concentrations were measured *via* the BCA Protein Assay Kit (Beyotime, Haimen, China). The proteins in each group were separated using CFAS Any KD PAGE gels (PE008; Shanxi ZHHC Biotechnology Co., Ltd., China). After electrophoresis, proteins were blotted onto polyvinylidene fluoride membrane (PVDF; Millipore) (200 mA, 1 h). They were then blocked with 5% milk dissolved in TBS-Tween (0.1%) for 1 h at 37°C and then incubated with a primary antibody against  $\alpha_{2A}$ -AR (1  $\mu$ g/mL dilution, ab85570; Abcam) and GAPDH (1:4000 dilution, 10494-1-AP; Proteintech, Rosemont, IL, USA) in blocking buffer overnight at 4°C. The membranes were washed with TBS-Tween (3 $\times$ 10 min) and incubated with a secondary antibody (1:5000 dilution, CoWin Biotech, Beijing, China) at room temperature for 2 h. The E-BLOT Touch Imager (e-BLOT, Shanghai, China) was used to capture the chemiluminescence. All bands of interest were quantified and normalized to their respective GAPDH bands.

### Statistical analysis

All data are presented as the mean  $\pm$  SD. Analyses of ABR threshold and DOPAE amplitudes were completed using a two-way analysis of variance (two-way ANOVA) followed by the Tukey-Cramer test. Analyses of  $\alpha_{2A}$ -AR expression at various stages were completed using a one-way analysis of variance (one-way ANOVA) followed by Dunnett's test. Analyses of  $\alpha_{2A}$ -AR expression in noise and control group were completed using a Student's *t*-test. All statistical analyses were performed using GraphPad Prism version 8.3.0 for Windows (GraphPad Software, San Diego, CA, USA). Significance was set as  $p < 0.05$ .

## Results

A rat model of NIHL was developed to investigate the expression of  $\alpha_{2A}$ -AR (Figure 1A), the ABR threshold was slightly higher in noise group at all frequencies tested than in the control group ( $n=10$ , two-way ANOVA,  $p < 0.0001$ ), meanwhile, the mean DOPAE amplitudes of the noise group were lower than that of the control group at all tested frequencies, with statistical significance ( $n=10$ , two-way ANOVA,  $p < 0.0001$ ) (Figure 1 B,C). Western blotting was used to investigate the expression of  $\alpha_{2A}$ -AR between two groups (Figure 1D). We found that the expression of  $\alpha_{2A}$ -AR significantly increased in rats exposed to noise compared with controls ( $n=10$ , Student's *t*-test,  $p < 0.05$ ). It suggested that the receptor plays an important role in NIHL. To investigate the distribution patterns of  $\alpha_{2A}$ -AR in the rat cochlea, we carried out immunofluorescence staining during postnatal development (P0, P7, P14, P21, and P28). We found that  $\alpha_{2A}$ -AR were predominantly immunoreactive in the HCs, SGNs, and cells of the SV from P0 to P28 in the rat cochlea (Figure 2, Supplementary Figure 1). The distributions of  $\alpha_{2A}$ -AR in the cochlea were predominantly immunoreactive in the plasma membrane and cytoplasm of HCs, SGNs, and cells of the SV at different developmental stages. In detail, we conducted triple-labeling using a marker of HCs and cells in the SV<sup>48</sup>, phalloidine, to observe whether  $\alpha_{2A}$ -AR was expressed in HCs and cells in the SV (Figures 3 and 4). Furthermore, we performed triple-labeling using an antibody against TUJ-1, which is a marker of SGNs, to determine whether  $\alpha_{2A}$ -AR were expressed in SGNs (Figure 5). No immunoreactivity was observed in the negative controls (Supplementary Figure 2). We quantified  $\alpha_{2A}$ -AR mRNA expression in the developing rat cochlea *via* qRT-PCR. As shown in Figure 6,  $\alpha_{2A}$ -AR mRNA reached a maximum level at P14 and P28 when compared to the P0 ( $n=6$ , one-way ANOVA,  $p < 0.05$ ). Western blotting revealed that  $\alpha_{2A}$ -AR proteins were present in the developing rat cochlea (Figure 7). We detected immunoreactive bands of 51 kDa corresponding to  $\alpha_{2A}$ -AR in cochlear tissue. GAPDH (35 kDa) was used as an internal control. Furthermore, we quantified  $\alpha_{2A}$ -AR protein expression levels at various stages relative to GAPDH as a control. Inconsistent with the qRT-PCR data, we found no significant differences in  $\alpha_{2A}$ -AR protein levels at the various stages when compared to the P0 ( $n=6$ , one-way ANOVA,  $p > 0.05$ ).

**Table 1.** Primers for real-time polymerase chain reaction

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH	GTCTTACCACCACGGAGAAGGC	ATGCCAGTGAGCTTCCCGTTCAGC
$\alpha_{2A}$ -AR	GGTAAGGTGTGGTGCGAGAT	CAGCGCCCTTCTTCTCTATG

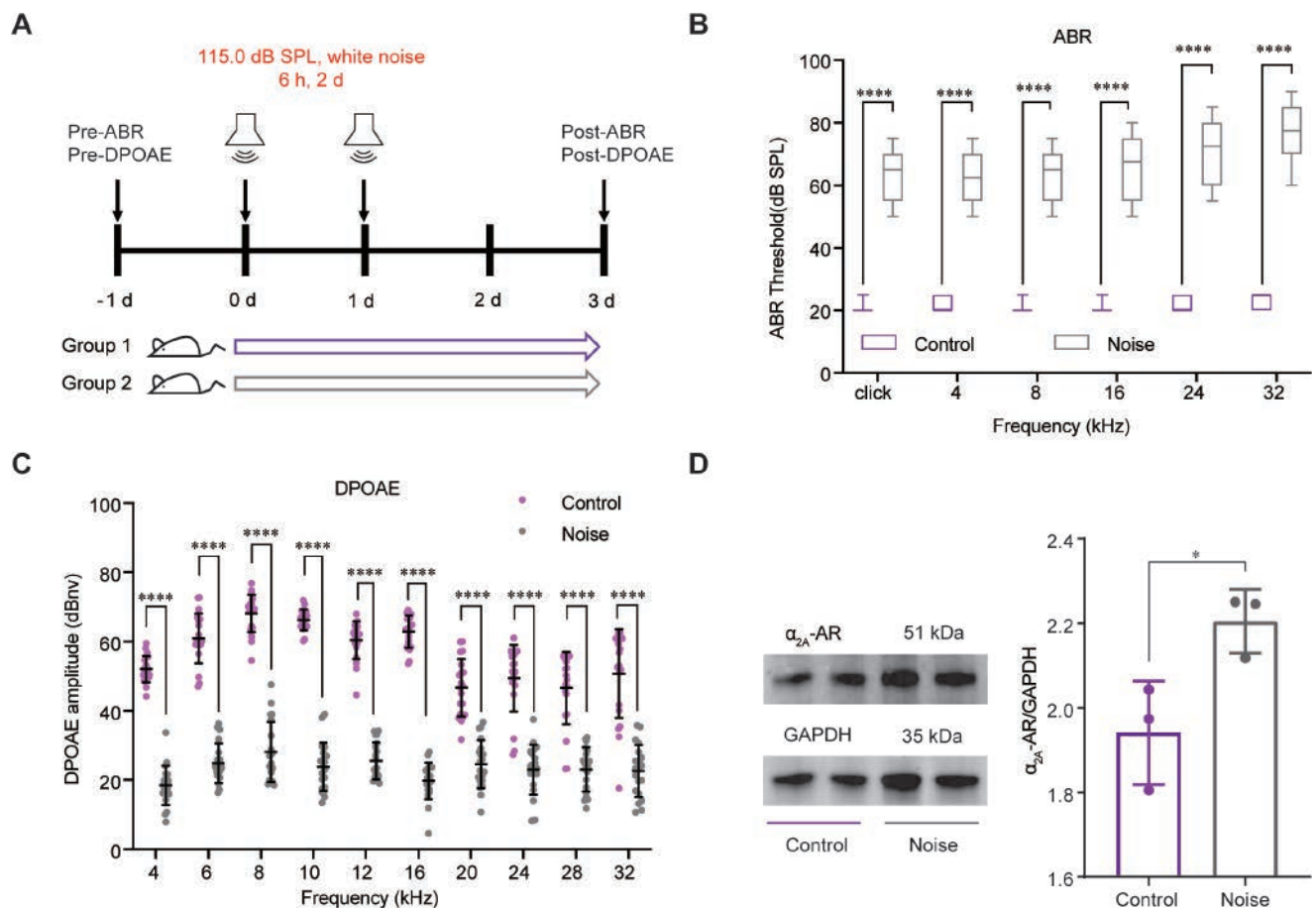
GAPDH, glyceraldehyde-3-phosphate dehydrogenase.



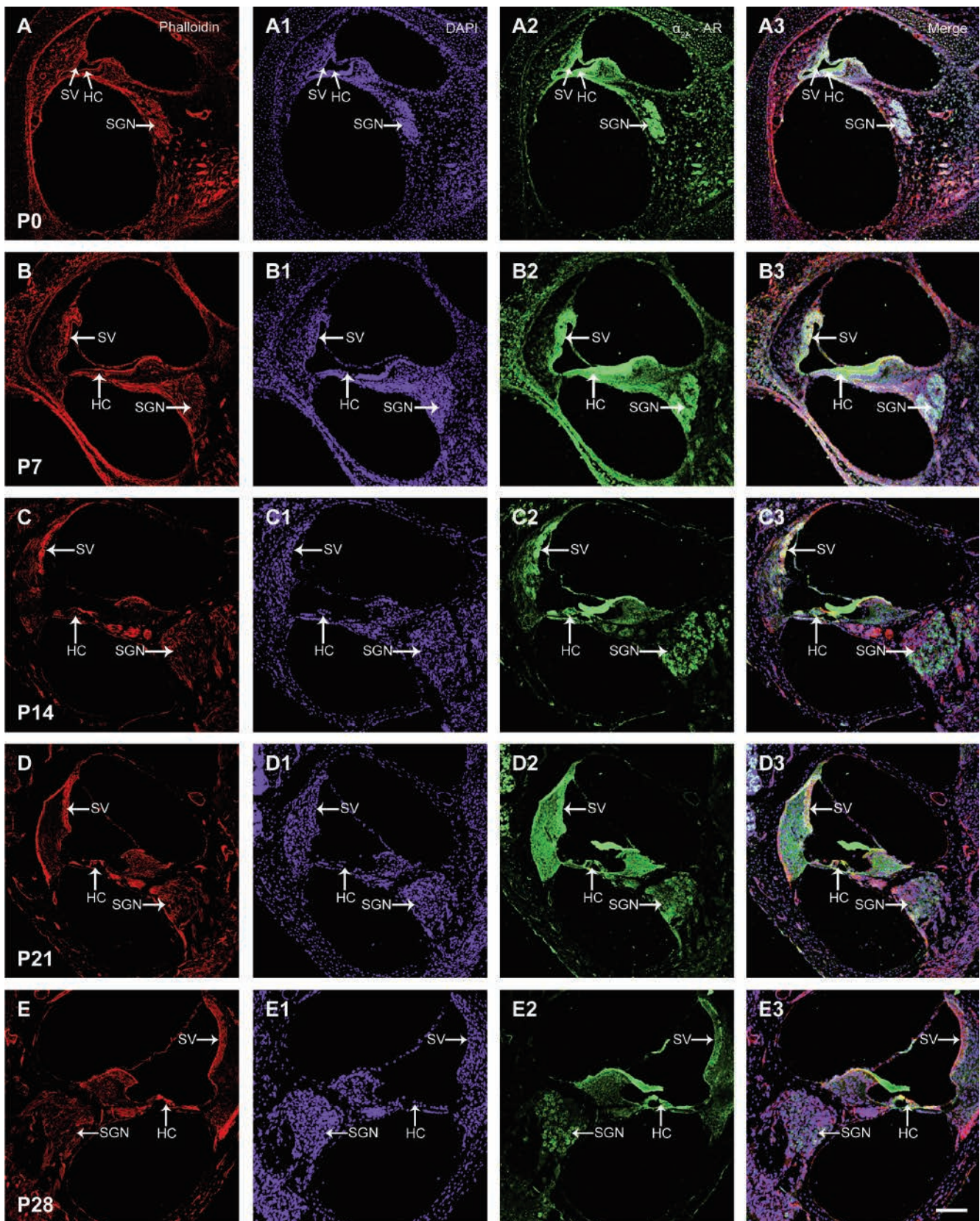
## Discussion

Organ of Corti, SGNs, and SV are regarded as three major cochlear structures that can independently degenerate and influence the degree of hearing loss.<sup>49,50</sup> The SNS projections into the inner ear have two main components:<sup>3,51,52</sup> a vessel-associated component and a vessel-independent component.<sup>4-6</sup> In pathological states, the SNS is associated with NIHL *via* CBF regulation, and the function of NE in CBF has been well established.<sup>53-60</sup> ARs also play a role in hearing disorders such as vertigo by regulating inner ear ion exchange.<sup>61</sup> Although both  $\alpha_1$ - and  $\alpha_2$ -ARs have been localized in the cochlea,  $\alpha_2$ -ARs is the predominant receptor in this region.<sup>29</sup> Furthermore, while a selective  $\alpha_1$ -antagonist increased CBF, a selective  $\alpha_2$ -antagonist,  $\beta$ -antagonist, and non-selective  $\alpha$ -antagonist had no effect on CBF.<sup>62</sup>  $\alpha_1$ -ARs have been localized in the chinchilla cochlea *via* the  $\alpha_1$ -AR antagonist [H]-prazosin.<sup>63</sup> These receptors may be related to the cochlear microvasculature, although the anatomical locations have not yet been determined. The spiral modiolar artery contains  $\alpha_{1A}$ -AR, which mediate vaso-

constriction at branch points.<sup>26</sup>  $\alpha_2$ -AR have been implicated in physiological responses and innervation in the organ of Corti.<sup>64</sup> NE may regulate auditory transmission, *via*  $\alpha_2$ -ARs, through suppression of the GABA response.<sup>30</sup> The HCs in the rat neonatal cochlea express all three  $\alpha_2$ -ARs subtypes.<sup>28</sup> The expression of  $\alpha_{2A}$ -,  $\alpha_{2B}$ -, and  $\alpha_{2C}$ -ARs has been confirmed in rat SGNs, and brimonidine acts directly on these receptors to protect the SGN.<sup>27,65</sup>  $\alpha_1$ -,  $\beta_1$ -, and  $\beta_2$ -ARs also have been immunohistochemically identified within the organ of Corti and SGNs.<sup>25</sup>  $\beta_1$ -AR play a role in regulating ion exchange in the SV.<sup>66</sup> Meanwhile,  $\beta_2$ -AR mRNA is present in the SV, and is implicated in the generation of gerbil nonstrial lateral wall tissues.<sup>37</sup> Isoproterenol, *via*  $\beta$ -ARs, increases endolymphatic hydrostatic pressure in variable ways in the vestibule and cochlea.<sup>67</sup> The signaling pathway of SNS regulation of peripheral auditory function is not yet clear. In addition, the location and role of these receptors are still unclear.<sup>36</sup> Here, we investigated the expression of  $\alpha_{2A}$ -ARs in the developing mammalian cochlea with the goal of supporting a theoretical explanation of the function of the SNS in auditory function. Our immunofluorescence data con-

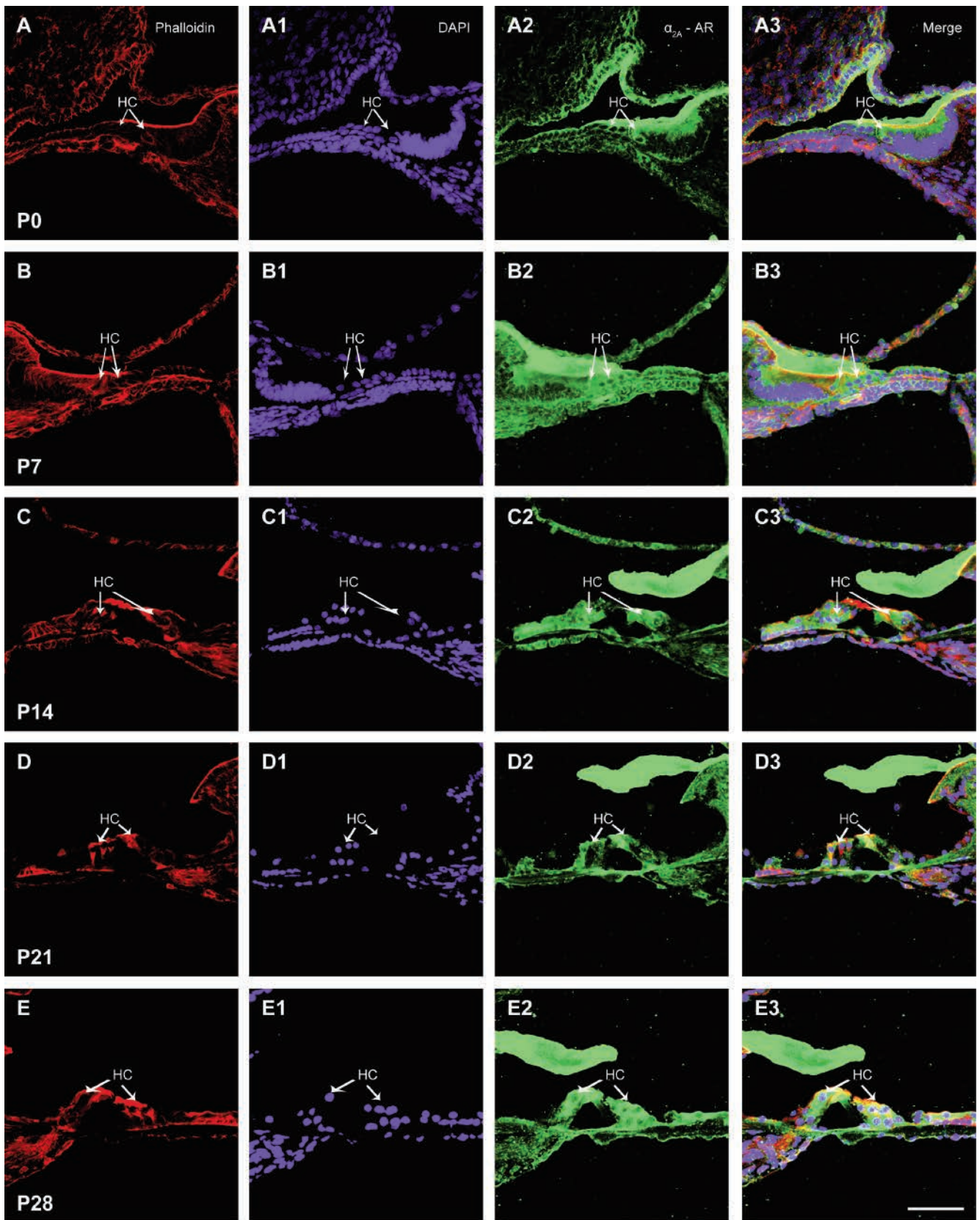


**Figure 1.** Traumatic noise exposure activates  $\alpha_{2A}$ -AR expression in rat cochlea. **A**) Schematic of the within-animal experimental design; animals were exposed to white noise at 115 dB SPL for 6 h per day during two consecutive days; ABR thresholds and DPOAE amplitudes were measured one-day pre-exposure and one-day post-exposure. **B**) Noise-induced ABR thresholds were significantly higher than control group at one-day post-noise; data were analyzed using a two-way analysis of variance (ANOVA) followed by Tukey *post-hoc* test (n=10 per group); \*\*\*\*p<0.0001. **C**) Noise-induced DPOAE amplitudes confirmed a significant decrease compared to control at one-day post-noise; data were analyzed using a two-way analysis of variance (ANOVA) followed by Tukey *post-hoc* test (n=10 per group); \*\*\*\*p<0.0001. **D**) Western blots using whole cochlear tissue homogenates reveal specificity for  $\alpha_{2A}$ -AR and show an increase in band densities for  $\alpha_{2A}$ -AR examined one day after the noise exposure compared with unexposed controls; data were analyzed using a Student's *t*-test (n=6 per group); \*p<0.05.

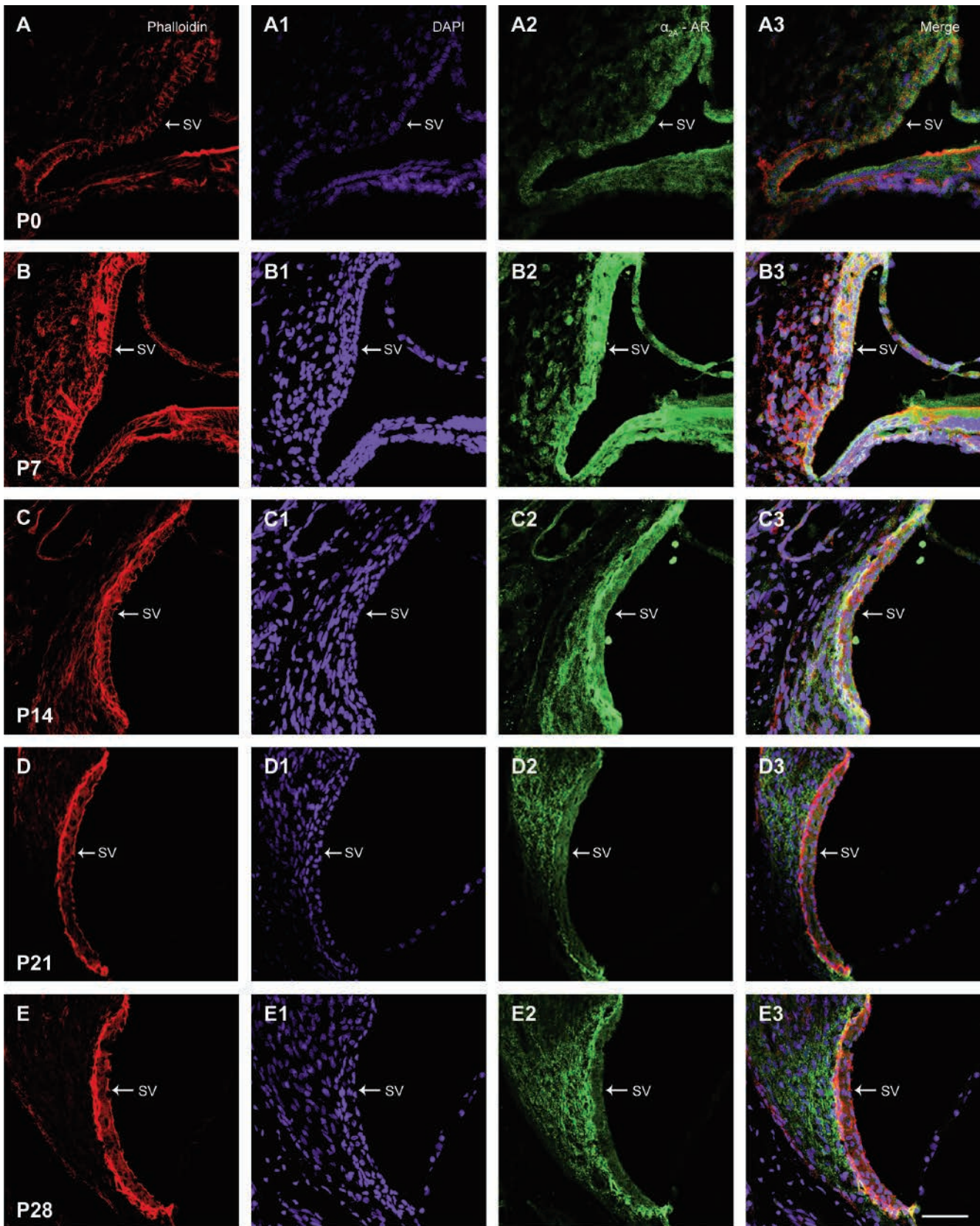


**Figure 2.** Immunofluorescence of  $\alpha_{2A}$ -AR within the developing cochlear tissues at various stages. **A-A3)** P0 group. **B-B3)** P7 group. **C-C3)** P14 group. **D-D3)** P21 group. **E-E3)** P28 group. Immunolabelling of a transverse section of developing cochlear tissues at various stages using phalloidine (red), DAPI (blue), and an antibody against  $\alpha_{2A}$ -AR (green).  $\alpha_{2A}$ -AR was mostly expressed in HCs, SGNs, and the SV. HCs, hair cells; SGNs, spiral ganglion neurons; SV, stria vascularis. Scale bars: 100  $\mu$ m.



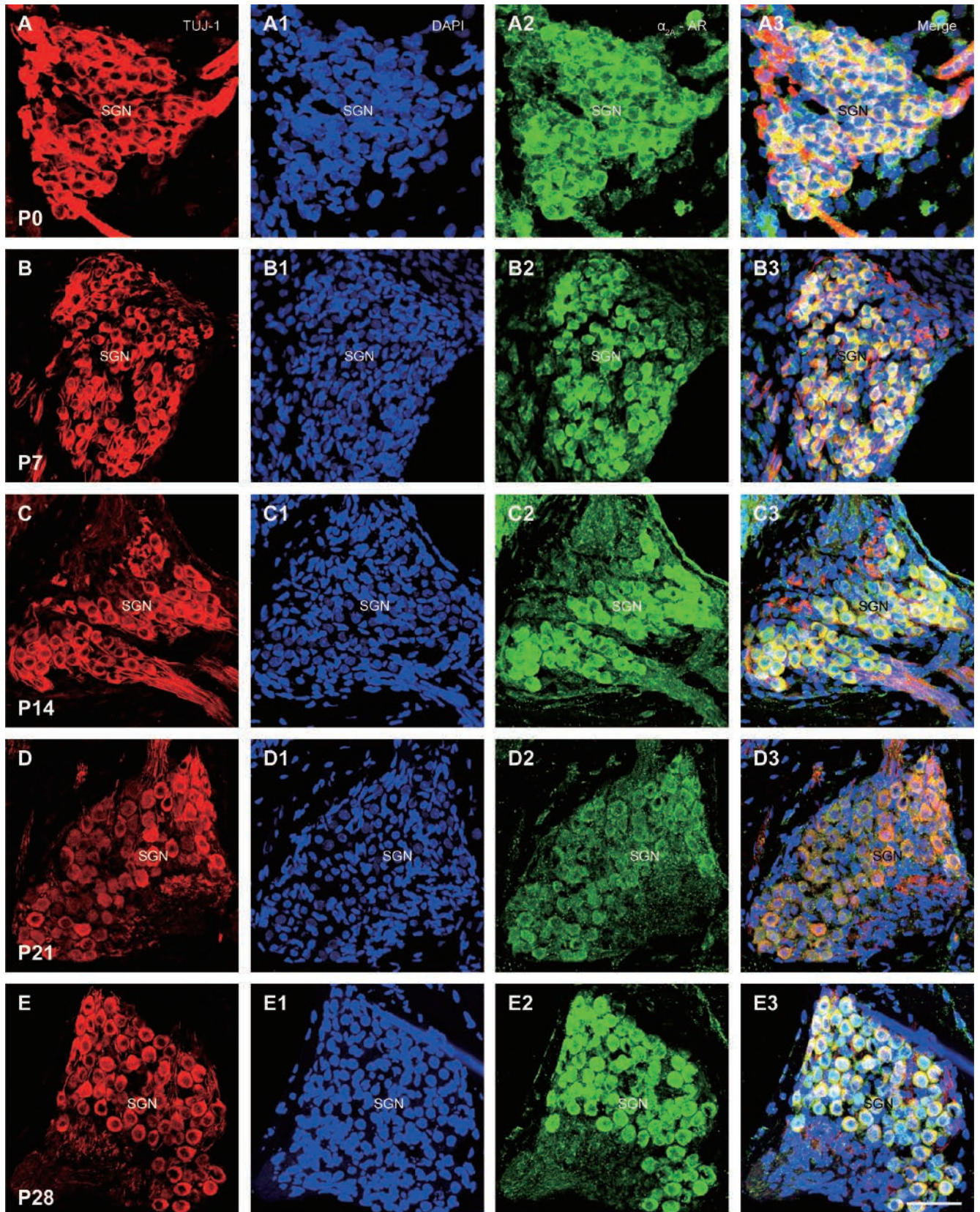


**Figure 3.** Immunofluorescence of  $\alpha_{2A}$ -AR in HCs at various stages. **A-A3)** P0 group. **B-B3)** P7 group. **C-C3)** P14 group. **D-D3)** P21 group. **E-E3)** P28 group. Co-immunolabelling *via* phalloidine (red), DAPI (blue), and an antibody against  $\alpha_{2A}$ -AR (green) focused on HCs in transverse sections of P0, P7, P14, P21, and P28 rat cochlea. HCs, hair cells. Scale bars: 50  $\mu$ m.



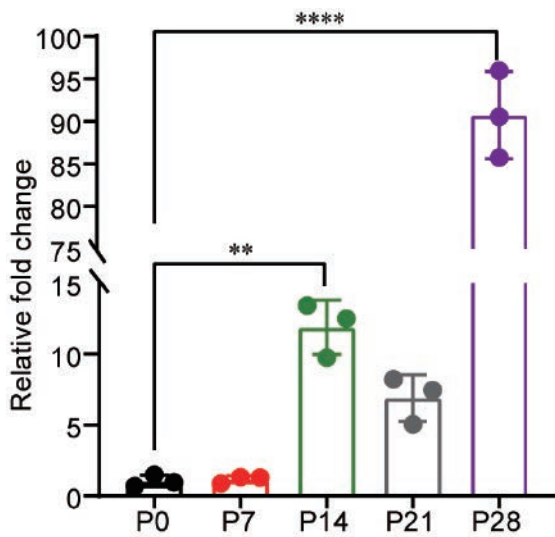
**Figure 4.** Immunofluorescence of  $\alpha_{2A}$ -AR in SV at various stages. **A-A3)** P0 group. **B-B3)** P7 group. **C-C3)** P14 group. **D-D3)** P21 group. **E-E3)** P28 group. Co-immunolabelling via phalloidine (red), DAPI (blue), and an antibody against  $\alpha_{2A}$ -AR (green) focused on SV in transverse sections of P0, P7, P14, P21, and P28 rat cochlea. SV, stria vascularis. Scale bars: 50  $\mu$ m.



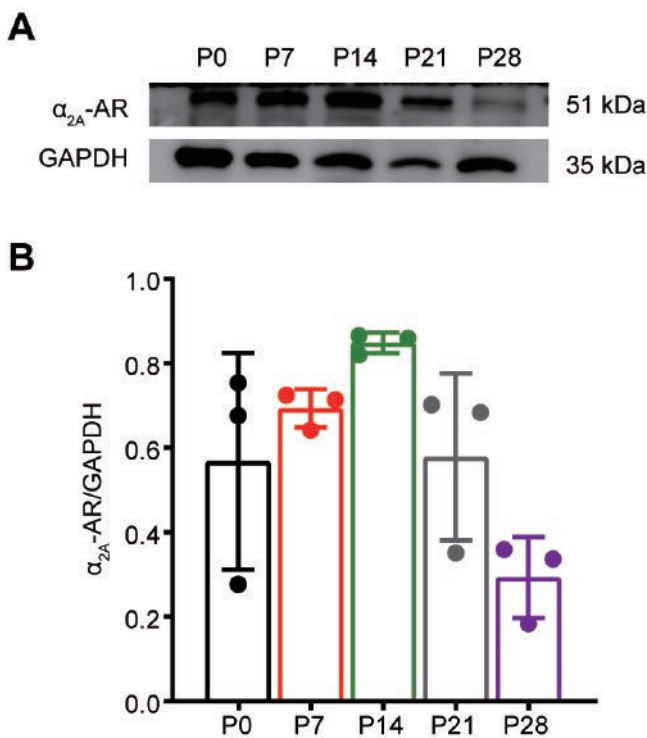


**Figure 5.** Immunofluorescence of  $\alpha_{2A}$ -AR in SGNs at various stages. **A-A3** P0 group. **B-B3** P7 group. **C-C3** P14 group. **D-D3** P21 group. **E-E3** P28 group. Co-immunolabelling *via* TUJ-1 (red), DAPI (blue), and an antibody against  $\alpha_{2A}$ -AR (green) focused on SGNs in transverse sections of P0, P7, P14, P21, and P28 rat cochlea. SGNs, spiral ganglion neurons. Scale bars: 50  $\mu$ m.





**Figure 6.** Expression of  $\alpha_{2A}$ -AR mRNA in the developing rat cochlea. qRT-PCR data showing significantly higher levels of  $\alpha_{2A}$ -AR in P14 and P28 rat cochlea compared with P0. \*\* $p < 0.005$ ; \*\*\*\* $p < 0.0001$ .



**Figure 7.** Developmental expression of  $\alpha_{2A}$ -AR in the postnatal rat cochlea. **A)** Representative immunoblots for  $\alpha_{2A}$ -AR and GAPDH in the rat cochlea during postnatal development. **B)**  $\alpha_{2A}$ -AR protein levels; no significant differences in expression levels were detected in the rat cochlea compared with P0; the band intensities for GAPDH demonstrate equal protein loading among the five stages.  $p > 0.05$ .

firming that  $\alpha_{2A}$ -AR are expressed in cochlear HCs, SGNs, and SV of the cochlea. Furthermore, we observed  $\alpha_{2A}$ -AR expression in the developing rat cochlea in terms of both mRNA and protein strengthens the credibility of our immunofluorescence results. Together, the findings of this study, therefore, allow us to speculate that NE might play a key role in the auditory functions of the cochlea via the receptor.

Cai *et al.* showed that the  $\alpha_2$ -ARs expressed in the rat cochlea and the vestibular labyrinth, however, they only selected the early postnatal period (P3 and P8) by immunohistochemistry.<sup>28</sup> In this study, we provide direct evidence for the expression patterns of  $\alpha_{2A}$ -AR in the rat cochlea. ARs were localized in different regions in the cochlea, indicating that these receptors may play a role in the processing of peripheral auditory information through multiple signaling pathways. The diverse distribution of this receptor in the mammalian cochlea also corresponds to the diversity of functions. Therefore, our data supported the results of previous studies.

In the current study, we selected  $\alpha_{2A}$ -AR to initially explore their expression in the developing rat cochlea, with the goal of providing a theoretical basis for subsequent studies on the distribution and function of other receptors. Data regarding the role of NE in auditory processing remain limited. Additional studies are needed with various experimental techniques to explore the role of NE and its receptors in peripheral auditory modulation in multiple species. Investigations of the expression and function of other ARs ( $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{1D}$ ,  $\alpha_{2B}$ ,  $\alpha_{2C}$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ), in addition to explorations of the precise molecular mechanisms underlying ARs modulation in the mammalian cochlea, it will increase our understanding of the role of the SNS in auditory function. In terms of clinical applications, the roles of catecholamines in cochlear functions such as responses to noise injury are topics worthy of future investigation. In summary,  $\alpha_{2A}$ -AR is predominantly expressed in the HCs, SGNs, and SV cells in the rat cochlea. Our findings indicate that NE might play a vital role in hearing function within the cochlea through its receptors.

### Acknowledgments

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