ONLINE SUPPLEMENT

ANG II-SALT HYPERTENSION DEPENDS ON NEURONAL ACTIVITY IN THE HYPOTHALAMIC PVN BUT NOT ON LOCAL ACTIONS OF TNF-α

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Immunohistochemistry Methods

Following transcardial perfusion, brains were removed, post-fixed in 4% PFA at 4^{o}C for 24 h, and transferred to 30% sucrose in 0.1 M PBS for at least 2 days. On the day of use, sections were rinsed 3 times in 0.1 M PBS, then incubated in 0.5% H_2O_2 in 0.1M PBS for 30 min followed by 1 h incubation in 10% normal goat serum (NGS) in 0.1 M PBS. Sections were permeabilized in 0.5% Triton X-100 for 10 min followed by overnight incubation with a monoclonal mouse anti-rat OX-42 antibody (1:100, Vector Laboratories) at 4^{o}C for 48 h. Sections were rinsed, incubated for 1 h with goat anti-mouse biotinylated secondary antibody (1:200, Vector Laboratories), and incubated in avidin-peroxidase conjugate for 30 min (Vector Laboratories). To control for non-specific staining, separate sections were incubated without primary antibody. To visualize immunoreactive product, sections were incubated with 0.05% 3,3'-diaminobenzidine hydrochloride in the presence of 0.003% H_2O_2. The reaction was terminated with an excess of distilled H_2O and sections were mounted on gelatin coated slides, dehydrated in graded concentrations of ethanol, cleared with xylene, air dried for 1-2 days, and cover-slipped with Cytoseal 60.

Results for PVN Histology

Injection sites marked with rhodamine microspheres were confined to an area encompassing the PVN as previously described by our laboratory[26]. Injections into NT rats (FigureS1) and HT rats (Figure S1) were typically centered on the rostral-caudal midpoint of PVN which included the dorsal cap region as well as magnocellular and parvocellular areas. Rhodamine beads did not enter the third ventricle or spread significantly to areas surrounding the PVN. Distributions of microinjected drugs other than minocycline and etanercept did not differ from those shown.

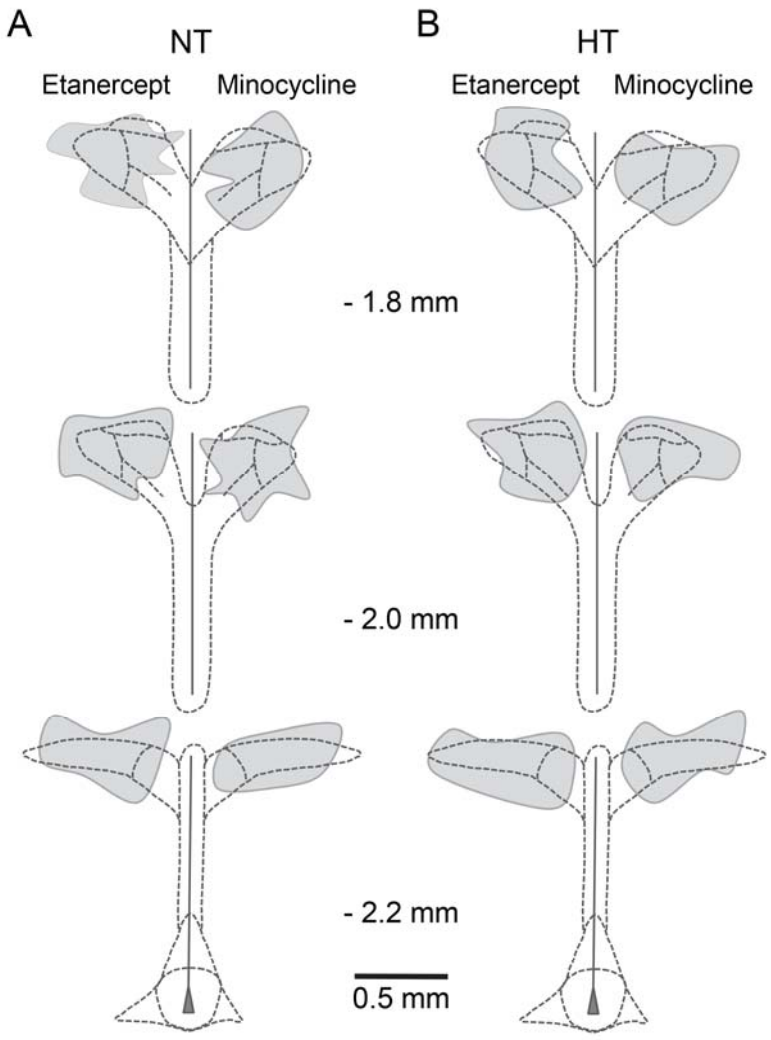

Figure S1: Summary of the distribution of PVN injected etanercept and minocycline among NT (**A**) and HT (**B**) rats. Injections delivered drugs within the area of PVN as previously indicated by our laboratory[26, 63]. Note that drugs were injected bilaterally into PVN, but distribution areas are shown unilaterally for clarity.