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RESEARCH ARTICLE

Figures and figure supplements

Antagonistic modulation of NPY/AgRP and POMC neurons in the arcuate nucleus by noradrenalin

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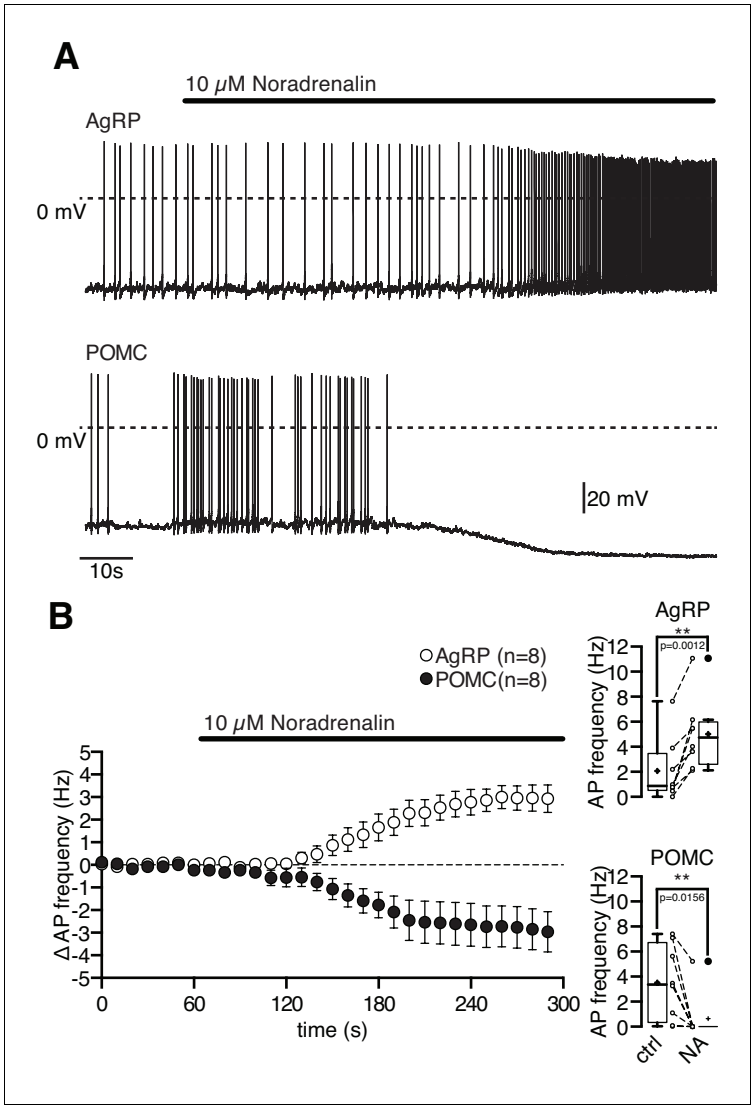

Figure 1. Noradrenalin differentially modulates functionally antagonistic NPY/AgRP and POMC neurons. NA (10 μM) excited orexigenic NPY/AgRP neurons and inhibited anorexigenic POMC neurons. Original recordings (**A**) and averaged responses (**B**) of NPY/AgRP (n = 8) and POMC neurons (n = 8) during NA application. The mean response is expressed as change in action potential frequency. The boxplots show the absolute change in action potential frequency for both neuron populations. **p<0.01, Wilcoxon matched pairs signed ranks test.

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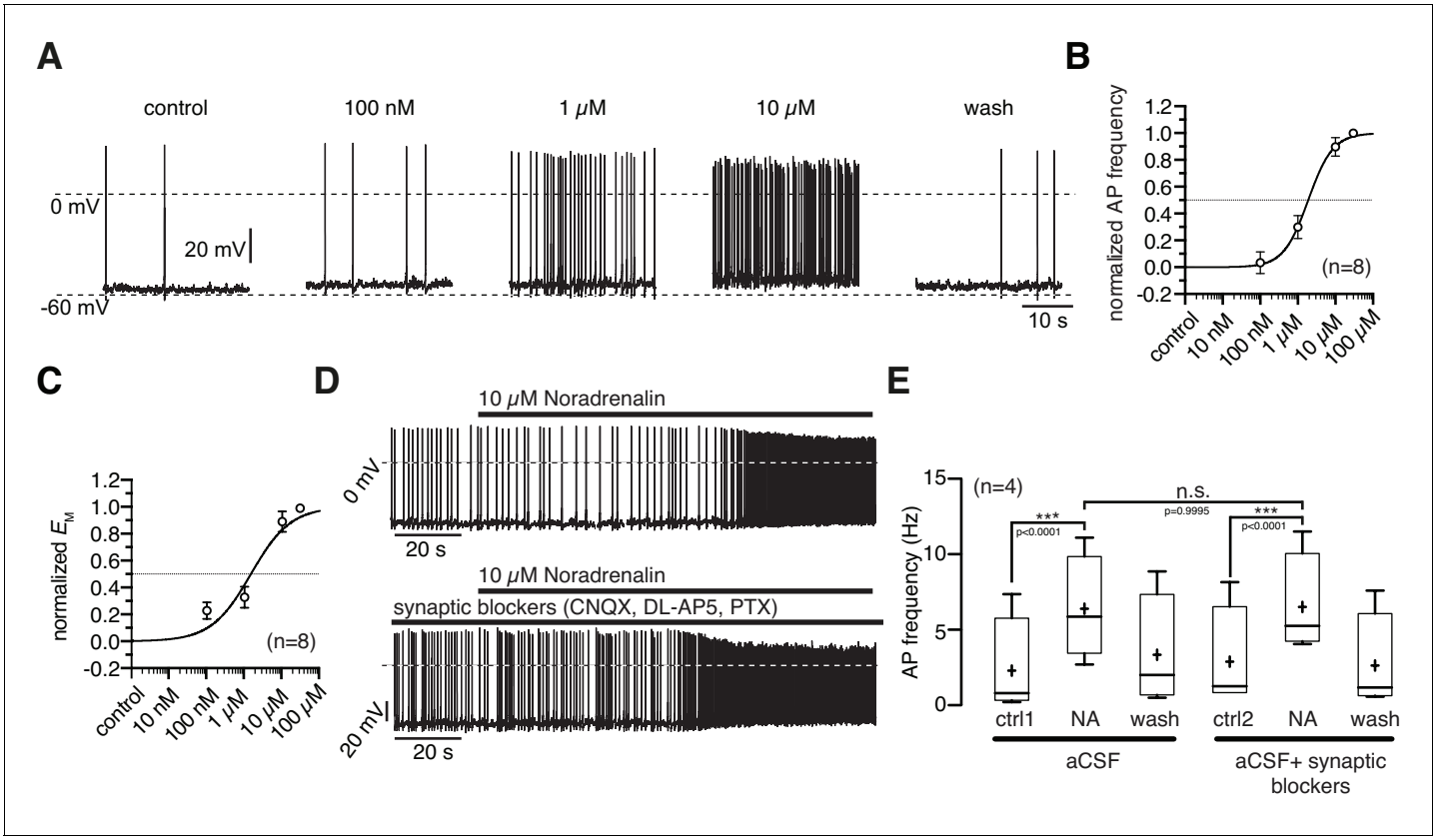

Figure 2. Noradrenergic modulation of NPY/AgRP neurons is concentration dependent. (**A**) Recording of a NPY/AgRP neuron demonstrating the effect of increasing NA concentrations. (**B**) and (**C**) *Concentration - response* relations showing the NA effect on action potential frequency (**B**) and membrane potential (**C**). The curves fit to a sigmoidal relation (***Equation 1***). NA had an EC_{50} of 1.9 μM (1.1–3.2 μM; n = 8) for the AP frequency and 1.5 μM (0.8–2.8 μM; n = 8) for the membrane potential, respectively. (**D**) and (**E**) The NA modulation of NPY/AgRP neurons is direct and not dependent on synaptic input. Original recording (**D**) and averaged effect on action potential frequency (**E**) showing that the NA effect on NPY/AgRP neurons is not changed when glutamatergic and GABAergic synaptic input is blocked. Experiments in (**E**) were performed consecutively with the same set of neurons (n = 8). Control 1 and control two refers to the different starting conditions, that is preincubation with or without synaptic blockers. ***p<0.001, one-way ANOVA with post hoc Tukey analysis.

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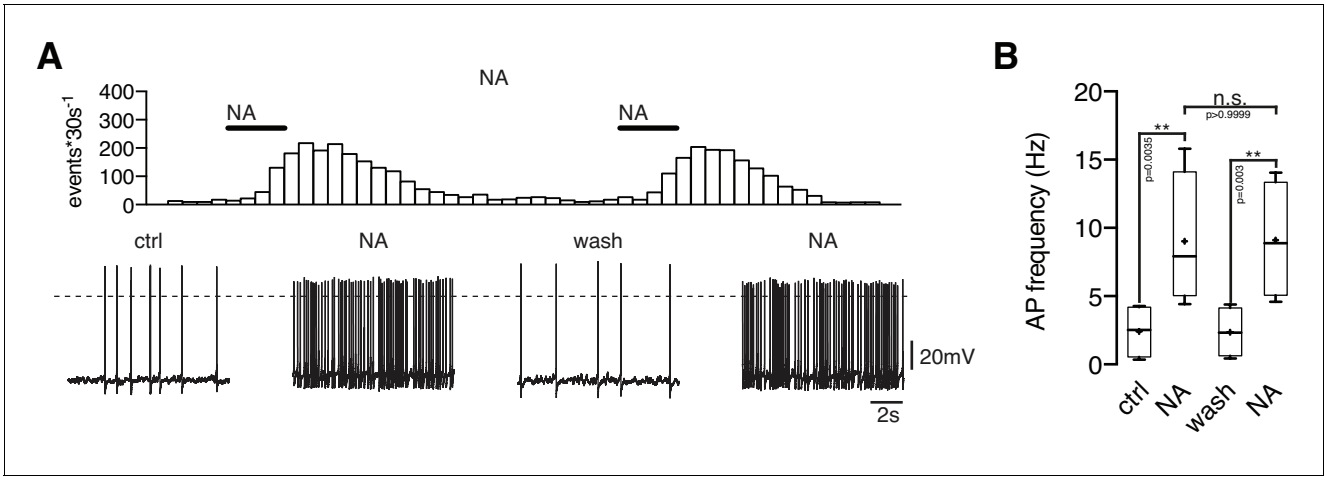

Figure 2—figure supplement 1. Repeated applications of noradrenaline do not cause desensitization. Original recording (**A**) of a NPY/AgRP neuron during two sequential applications of NA (10 µM) and averaged NA responses (**B**) on action potential frequency showing that there is no desensitization of the first application prior to the second treatment. n values are given in brackets. **p<0.01; one-way ANOVA with post hoc Tukey analysis.
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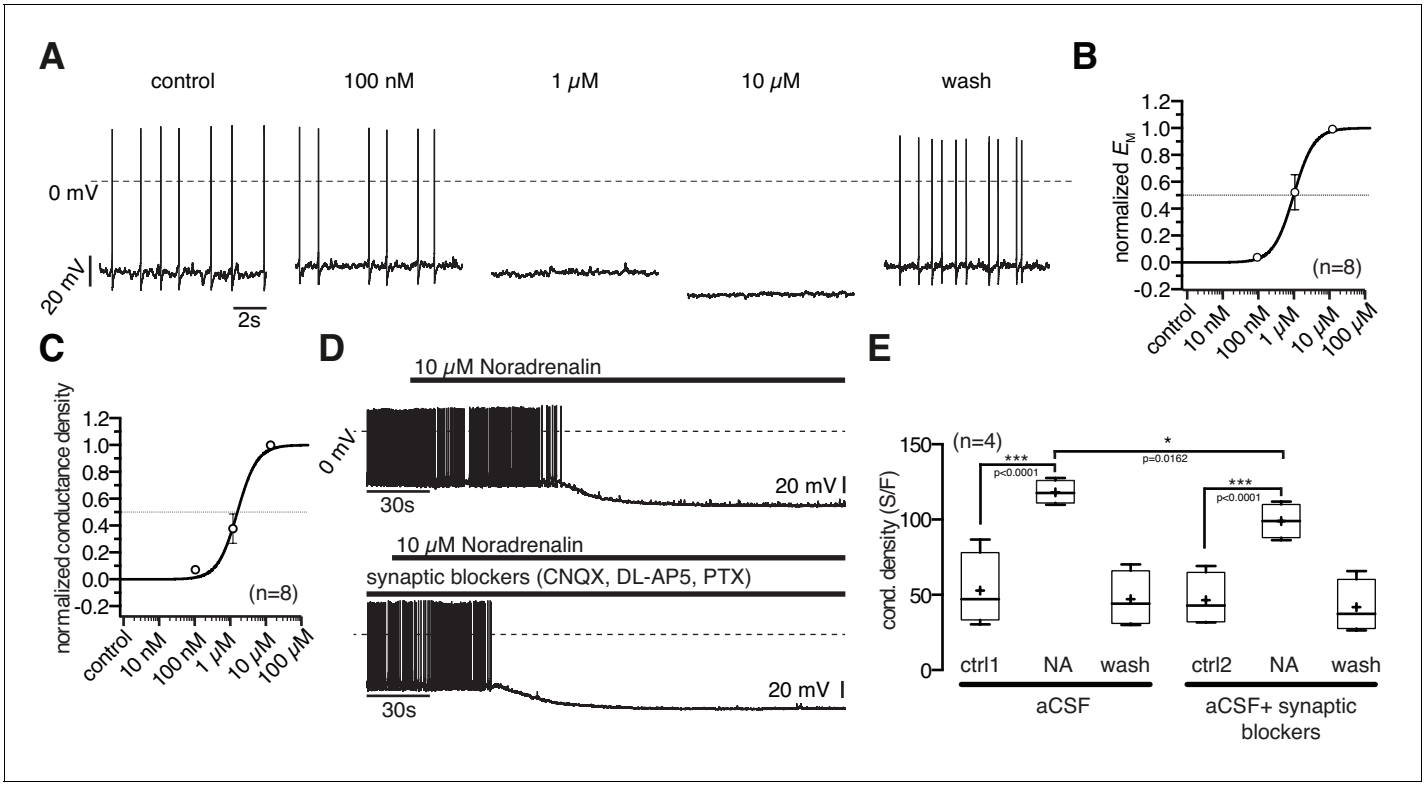

Figure 3. Noradrenergic modulation of POMC neurons is concentration dependent. (**A**) Recording of a POMC neuron demonstrating the effect of increasing NA concentrations. (**B**) and (**C**) *Concentration - response* relations showing the NA effect on membrane potential (**B**) and conductance density (**C**). The curves are fits to a sigmoidal relation (***Equation 1***). NA had an EC_{50} of 0.9 μM (0.6–1.5 μM; n = 8) for the membrane potential and 1.3 μM (1.0–1.9 μM; n = 8) for the conductance density, respectively. (**D**) and (**E**) The NA modulation of POMC neurons is direct and not dependent on synaptic input. Original recording (**D**) and averaged effect on conductance density (**E**) showing that the NA effect on POMC neurons is not changed when glutamatergic and GABAergic synaptic input is blocked. Experiments shown in (**E**) were performed consecutively with the same set of neurons (n = 4). Control 1 and control two refers to the different starting conditions, that is pre-incubation with or without synaptic blockers. **p<0.01; ***p<0.001; one-way ANOVA with post hoc Tukey analysis.

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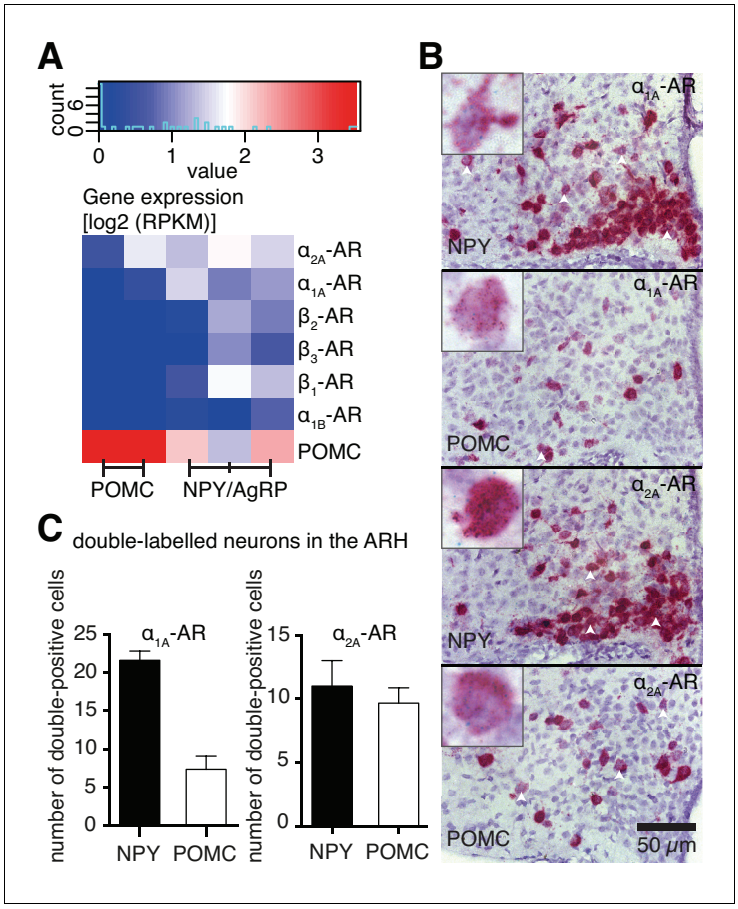

Figure 4. Cell type-specific expression of ARs. (**A**) Log expression levels of adrenergic receptor genes in Pomc- and Npy-expressing cell populations. Deeper red colours indicate higher expression levels in the respective cell population. RPKM: reads per kilobase per million mapped reads. (**B**) Images from RNA in situ hybridizations against *Adra1a* (upper panels, green dots) and *Adra2a* (lower panels, green dots) in *Npy*- and *Pomc*- expressing (red) neurons. White arrowheads indicate doubly-labeled cells. Higher magnification indexes are representative of doubly-labeled cells. (**C**) Quantification of double positive cells for *Adra1a* and *Adra2a* in *Npy*- and *Pomc*-expressing neurons.

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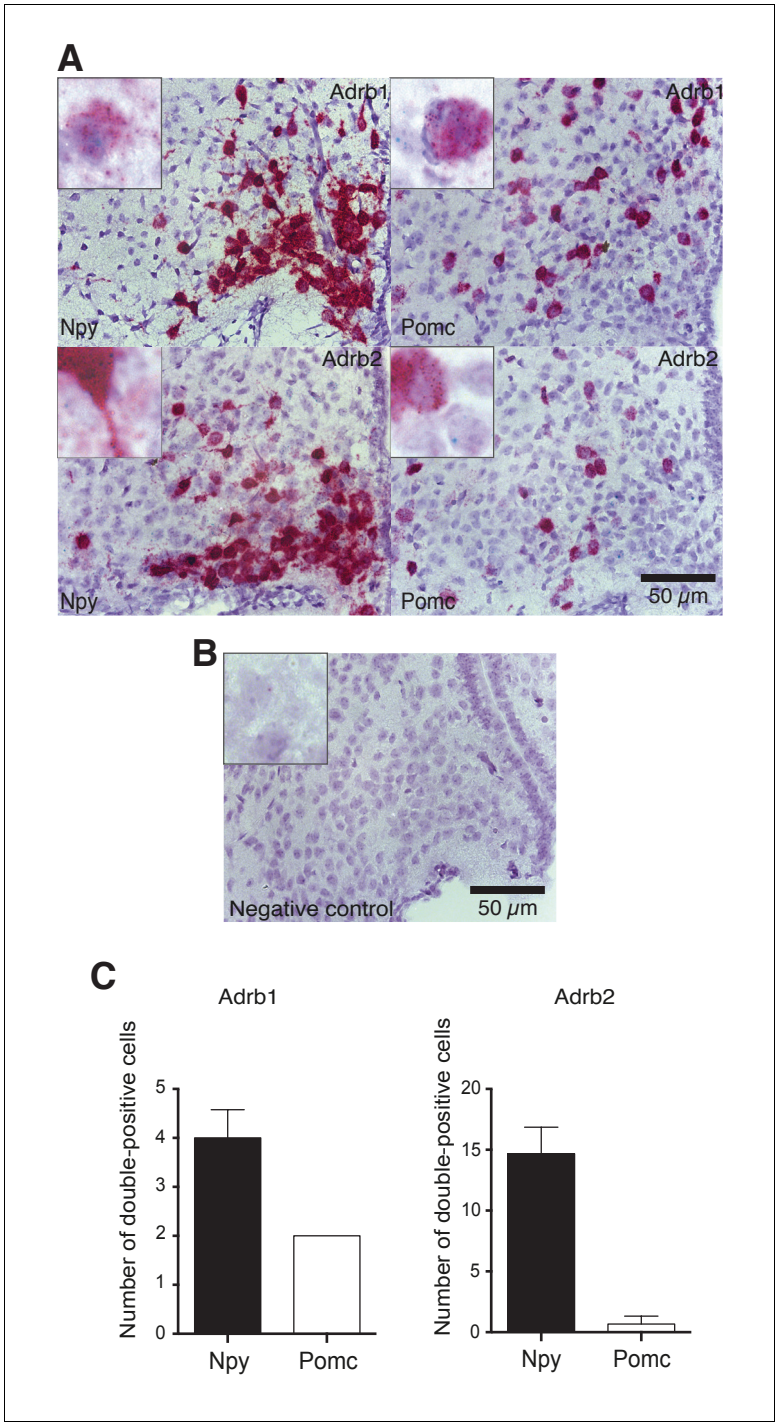

Figure 4—figure supplement 1. Images (**A**,**B**) and quantification (**C**) of RNA in situ hybridizations against Adrb1 (upper panels, green dots) and Adrb2 (lower panels, green dots) in Npy- and Pomc- expressing (red) neurons. Higher magnification indexes are representative of doubly-labeled cells. Negative control: probe against E. coli gene DapB.

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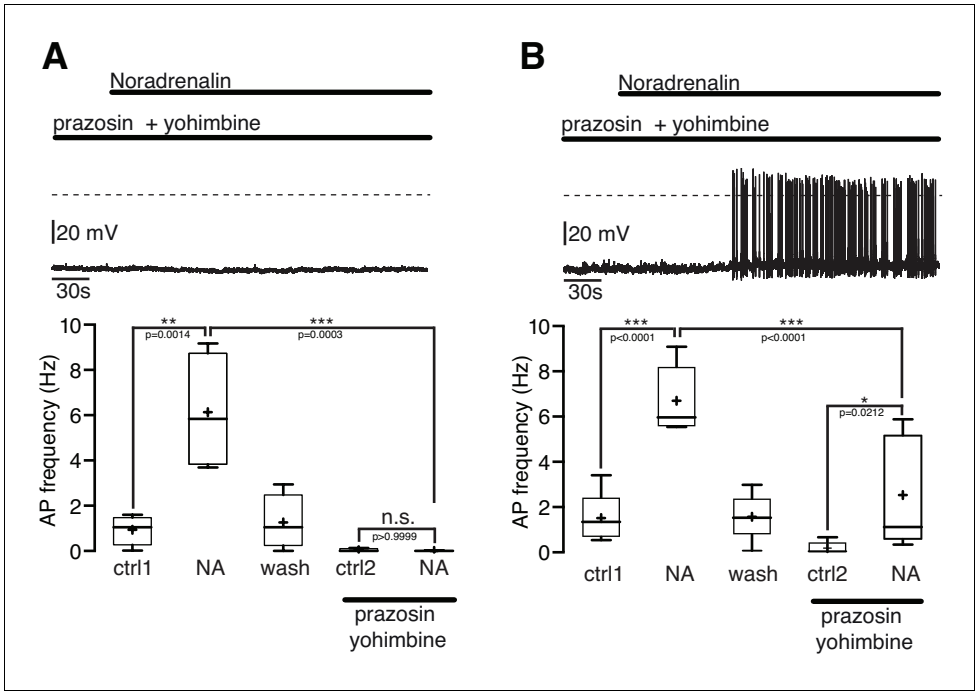

Figure 5. The excitatory noradrenergic effect on NPY/AgRP neurons is predominantly mediated by α_{1A}-ARs. (**A**) and (**B**) In 44% (4 out of 9) of the NPY/AgRP neurons the NA (10 µM) effect was completely blocked by the α_1-AR antagonist prazosine (5 µM) and α_2-AR antagonist yohimbine (5 µM) (**A**), while in 56% (5 out of 9) of the NPY/AgRP neurons the NA effect was markedly reduced (~60%) but not completely blocked (**B**). The series of experiments shown in (**A**) and (**B**) were each performed consecutively with the same set of neurons. n values are given in brackets. Control 1 and control two refers to the different starting conditions, that is pre-incubation with different AR antagonist. **p<0.01; ***p<0.001; one-way ANOVA with post hoc Tukey analysis.

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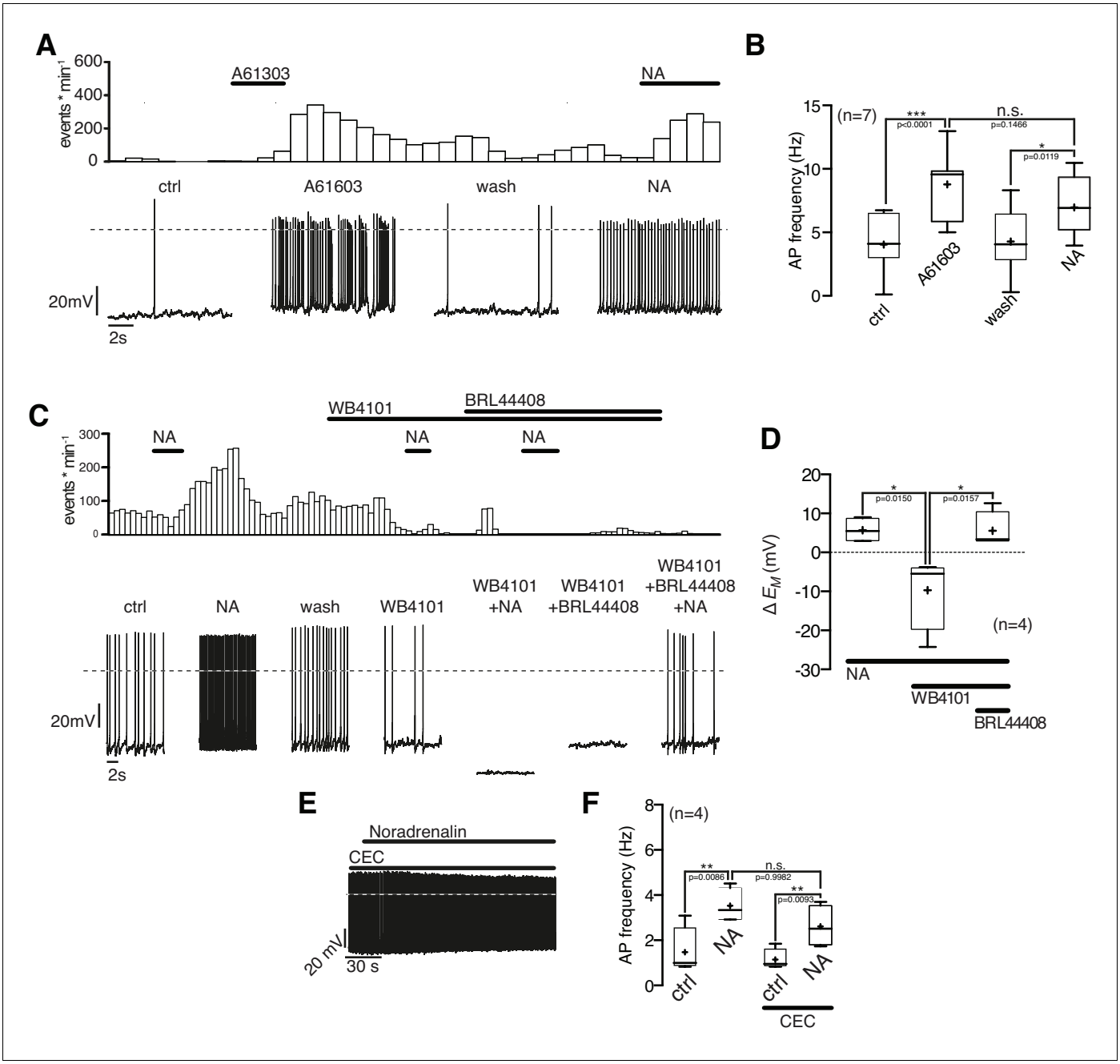

Figure 6. NPY/AgRP neurons are excited by α_{1A}-AR and inhibited by α_{2A}-AR when α_{1A}-AR are blocked. (**A**) Rate histogram with its original recording showing that the α_{1A}-AR agonist A61603 (1 μM) had similar excitatory effects on action potential frequency (**B**) as NA. (**C**) and (**D**) The selective α_{1A}-AR antagonist WB4101 (100 nM) blocked the excitatory NA effect. In the presence of WB4101 NA had an inhibitory effect on the membrane potential, which was eliminated by the α_{2A}-AR antagonist BRL44408 (10 μM). (**E**) The α_{1BD}-AR blocker CEC (100 nM) did not change the NA effect on NPY/AgRP neurons. *p<0.05; **p<0.01; ***p<0.001; one-way ANOVA with posthoc Tukey analysis.

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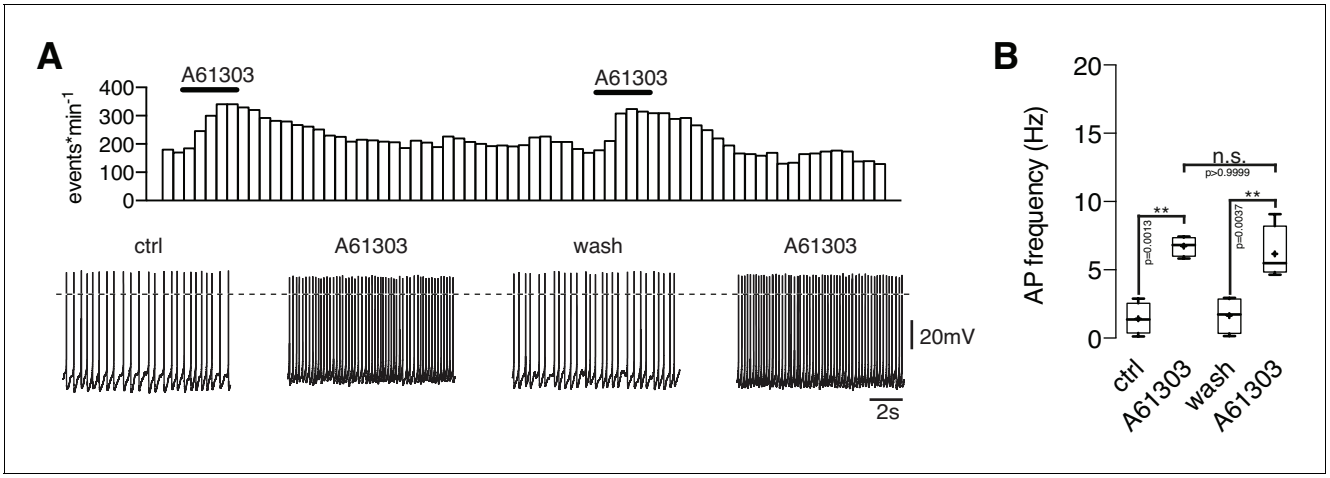

Figure 6—figure supplement 1. Repeated applications of noradrenaline do not cause desensitization. Original recording (**A**) of a NPY/AgRP neuron during two sequential applications of A61603 (1 μM) and averaged A61603 responses (**B**) on action potential frequency showing that that there is no desensitization of the first application prior to the second treatment. n values are given in brackets. **p<0.01; one-way ANOVA with post hoc Tukey analysis.

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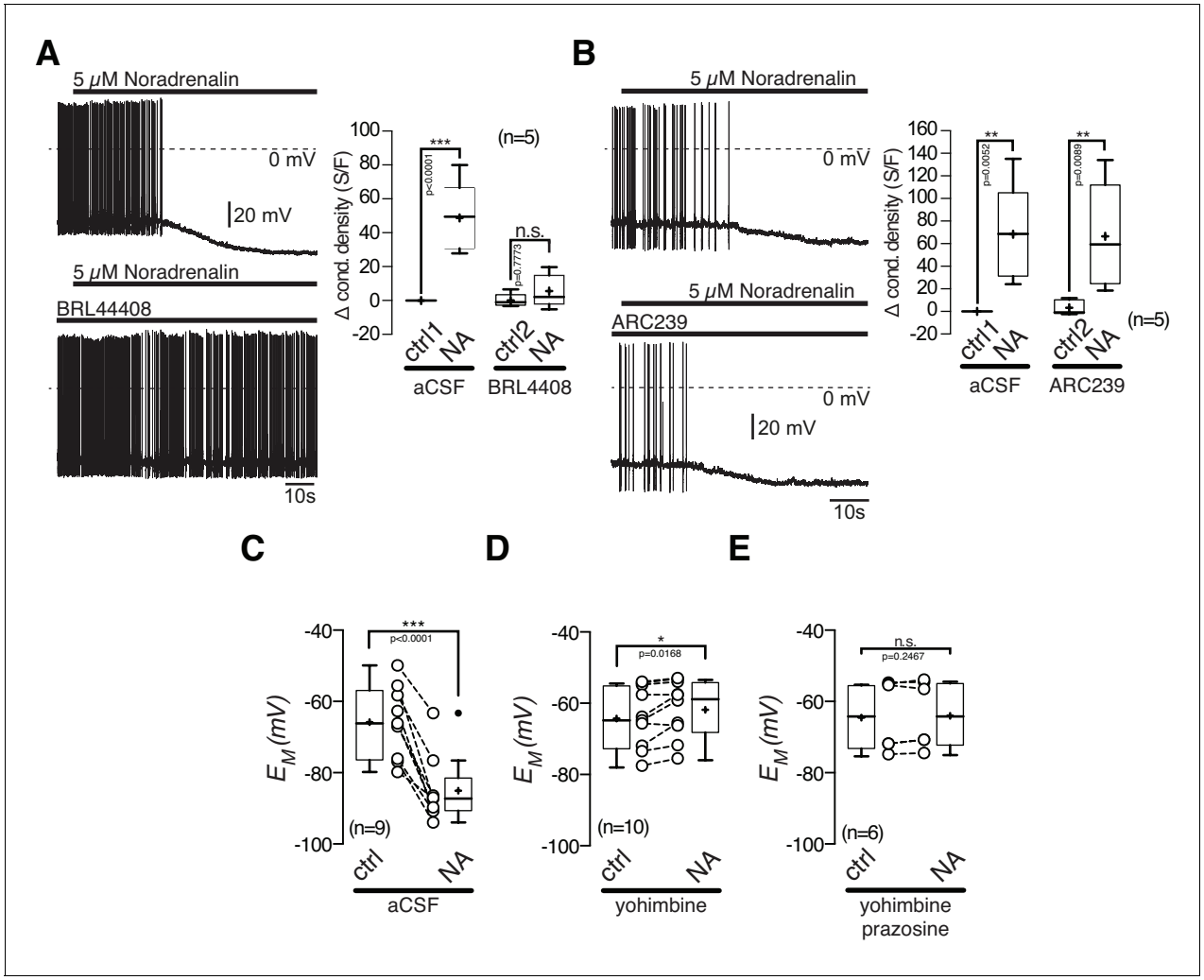

Figure 7. The inhibitory noradrenalin effect on POMC neurons is mediated by α_{2A}-AR. (**A**) and (**B**) The α_{2A}-antagonist BRL 44408 (10 μM) blocked the inhibitory NA (5 μM) effect (**A**), while the α_{2A}-antagonist ARC239 (1 μM) did not affect the NA action (**B**). (**C–E**) Inhibitory NA effect (**C**). Blocking α_2-AR by yohimbine revealed an excitatory NA effect (**D**), which was blocked by the α_1-AR antagonist prazosine (**E**). The series of experiments shown in (**A**) and (**B**) were each performed consecutively with the same set of neurons. Control 1 and control two refers to the different starting conditions, that is preincubation with different AR antagonist. n values are given in brackets. *p<0.05; **p<0.01; ***p<0.001. (**A**) and (**B**) one-way ANOVA with post hoc Tukey analysis; (**C–E**) paired t-test.

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