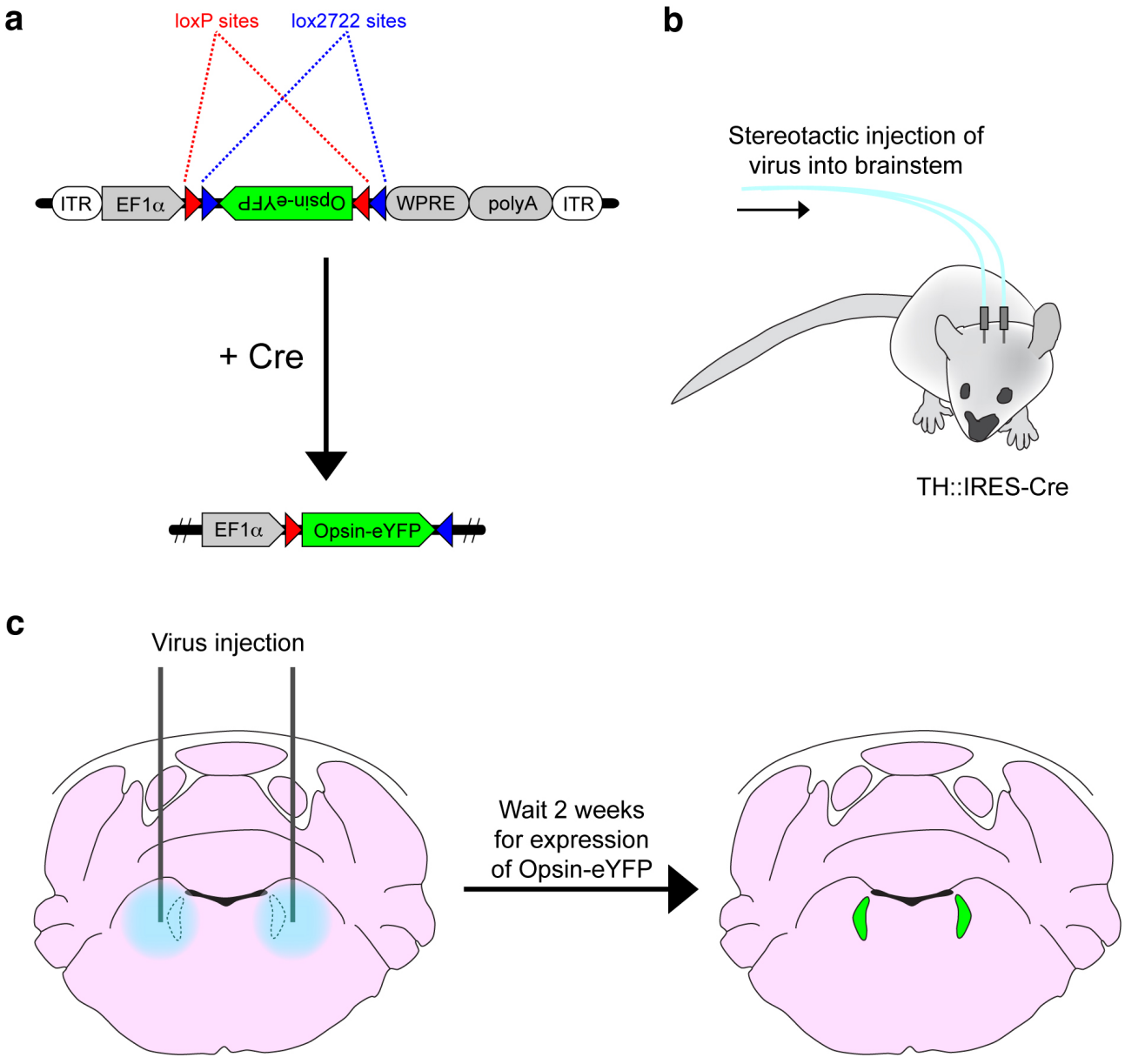
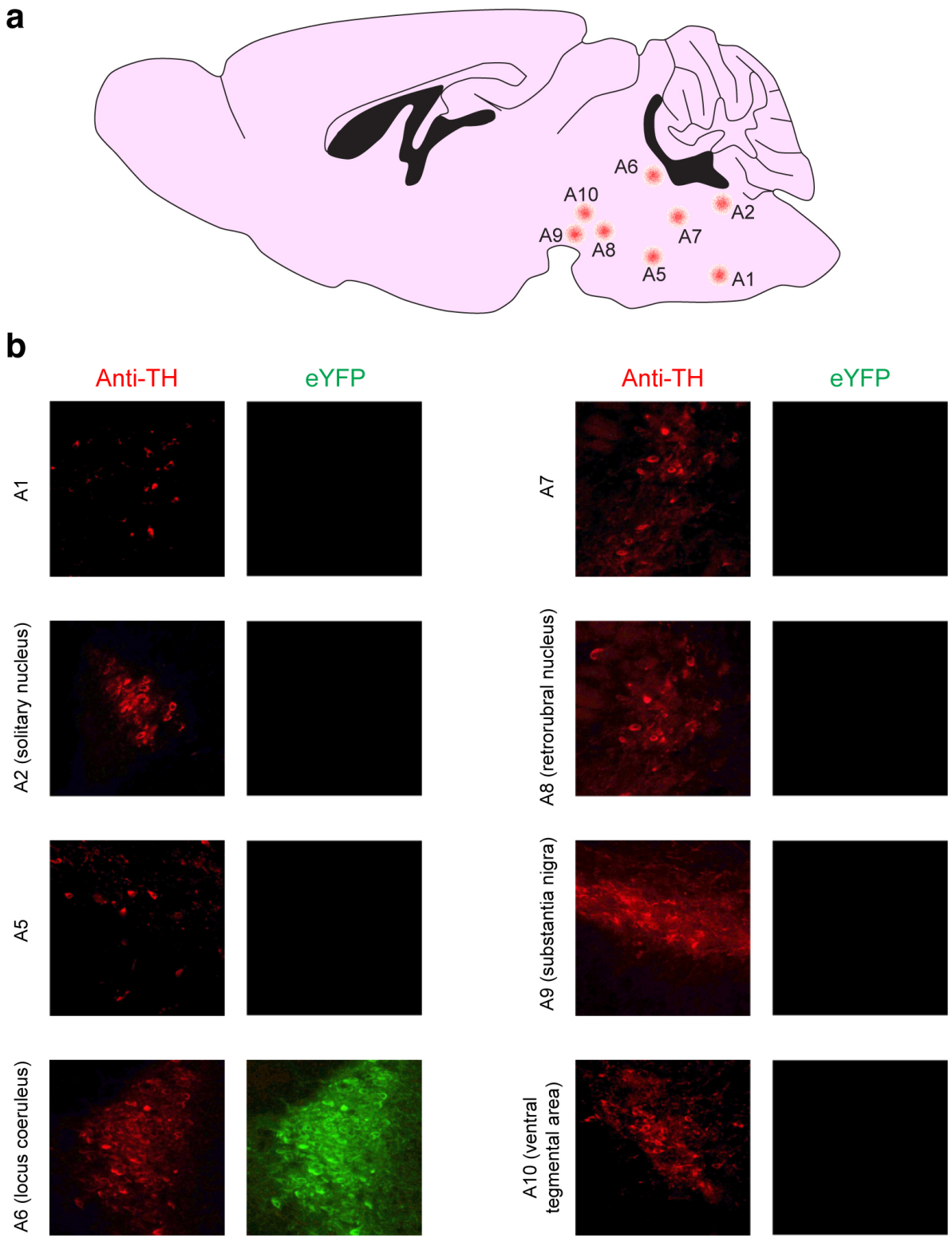
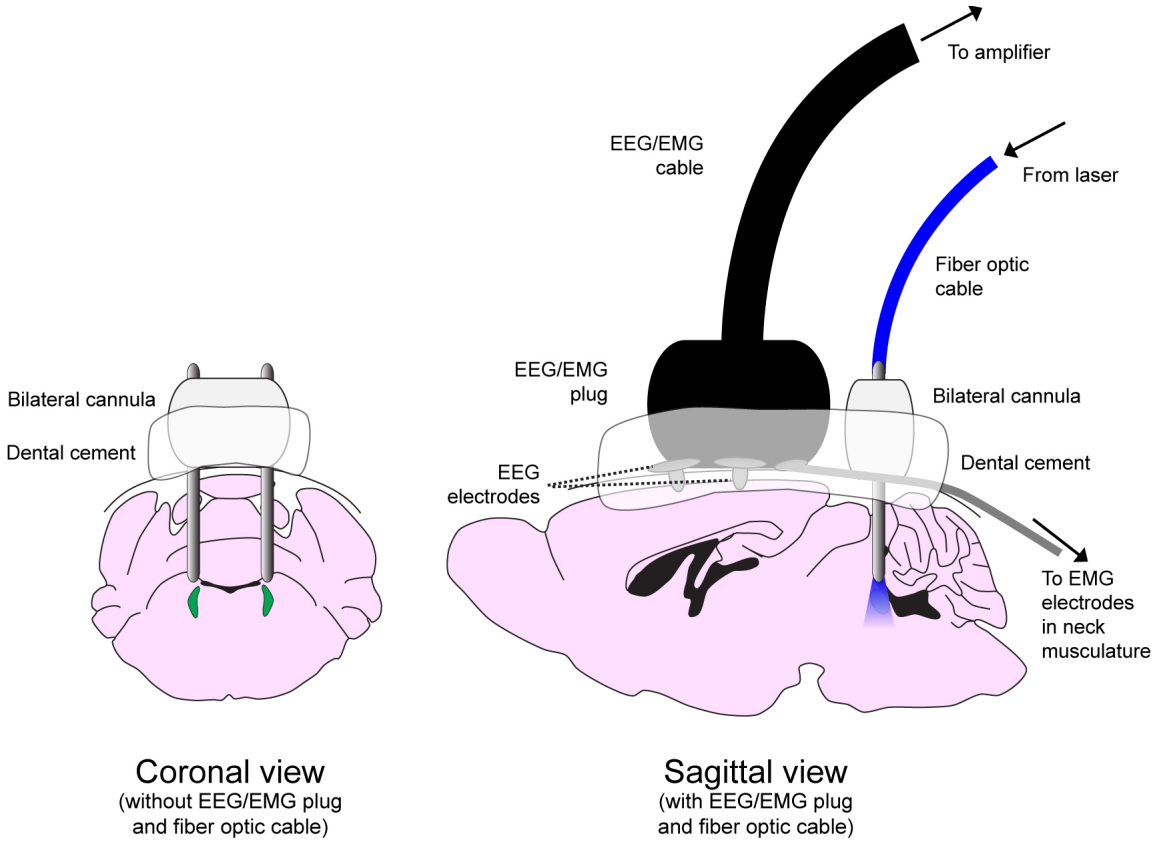
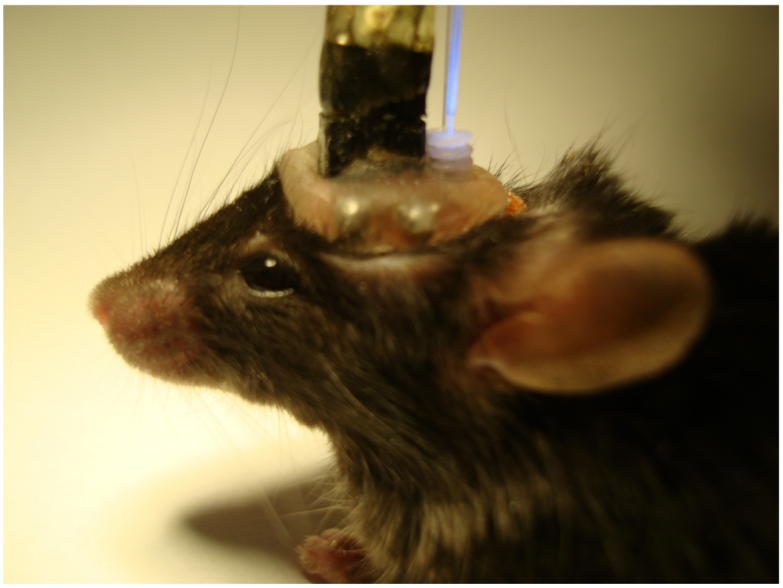


Supplementary Figure 1. Genetic strategy to target locus coeruleus neurons. **(a)** We used a double-floxed inverted open reading frame (DIO) construct within a recombinant adeno-associated viral vector (rAAV) to deliver optogenetic transgenes to locus coeruleus neurons. Our optogenetic transgenes (Opsin-eYFP) included eNpHR-eYFP, ChR2-eYFP, or eYFP alone. In the absence of Cre recombinase, the coding sequences for these transgenes reside in the opposite orientation. However, upon Cre-lox mediated recombination, the transgenes flip into the correct orientation so that efficient mRNA and protein expression can occur. **(b)** High-titer rAAV was stereotaxically injected into the brainstem of TH::IRES-Cre knockin mice in which Cre recombinase is specifically expressed in cells that express tyrosine hydroxylase. Thus, although the rAAV infects multiple neuronal subtypes in the brain, only tyrosine hydroxylase-positive cells in the local site of injection express transgenes in the correct orientation. **(c)** rAAV was injected just lateral to the locus coeruleus (anteroposterior, –5.45 mm; mediolateral, +/–1.28 mm; dorsoventral, 3.65 mm). Experiments began two weeks after injection to allow for efficient gene expression.

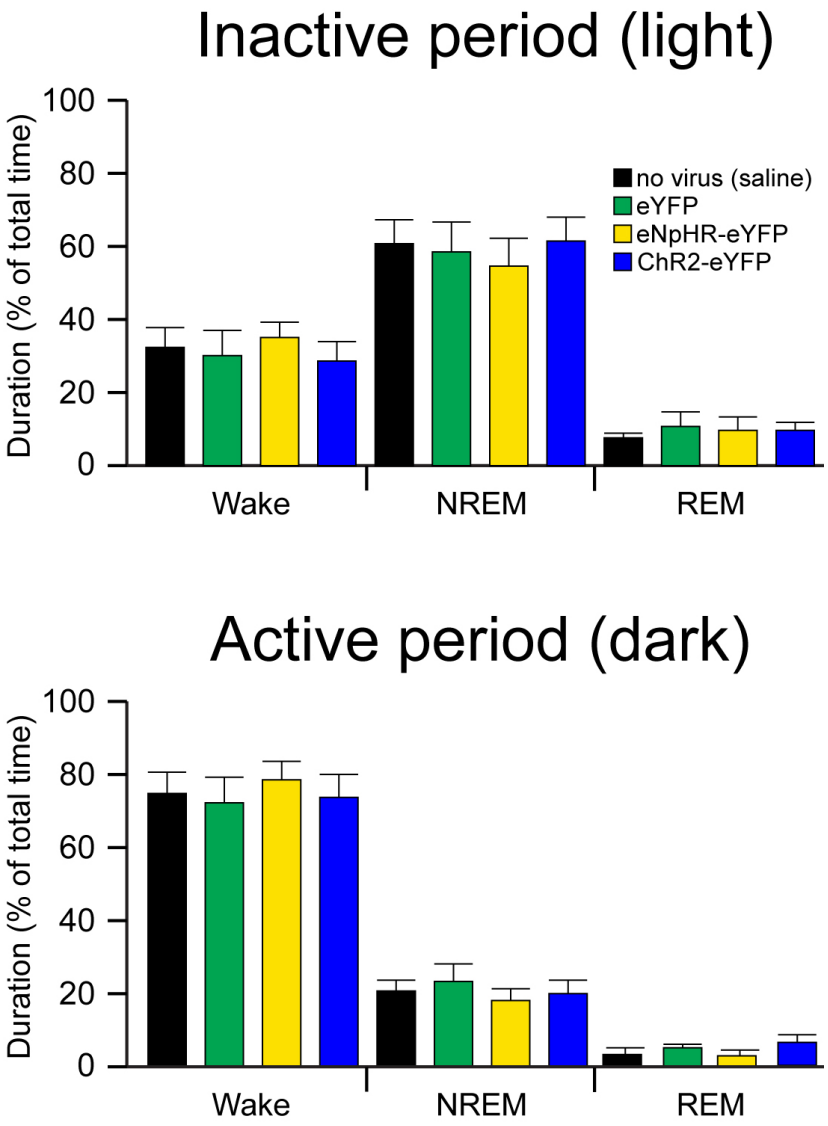


Supplementary Figure 2. Transgene expression in brainstem noradrenergic and dopaminergic nuclei. **(a)** Sagittal profile of the mouse brain depicting the locations of brainstem noradrenergic and dopaminergic nuclei. **(b)** Co-expression of tyrosine hydroxylase (TH) immunoreactivity (red), and viral eYFP expression (green) in brainstem noradrenergic and dopaminergic nuclei labeled in (a).

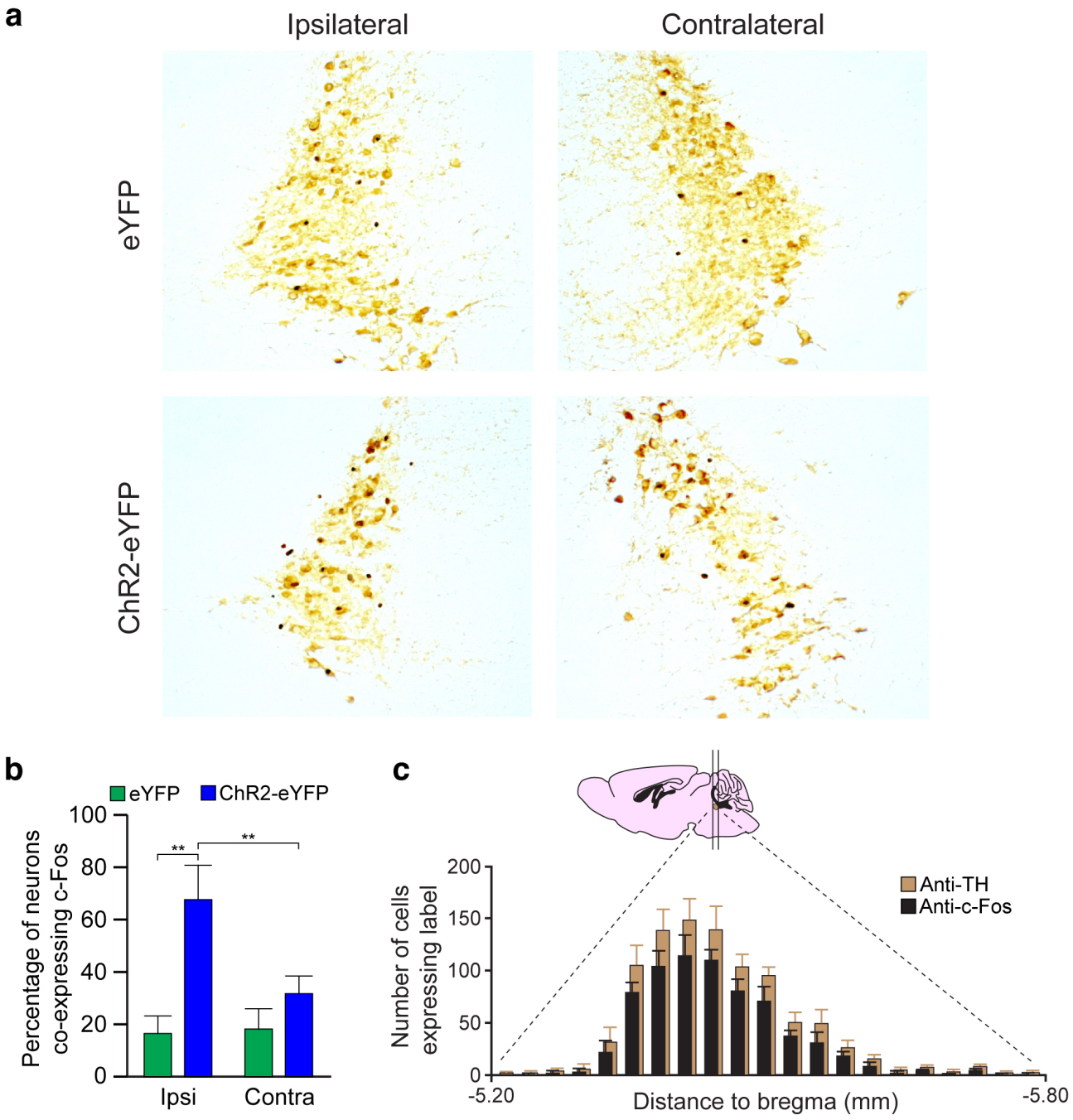




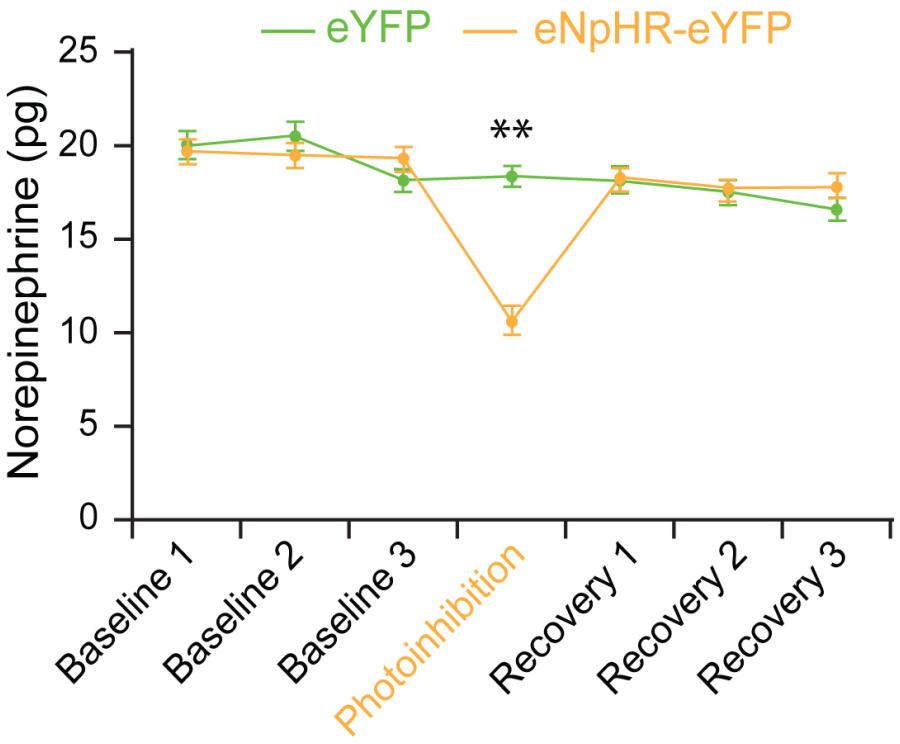
Supplementary Figure 3. Surgical implantation of bilateral cannulae and EEG/EMG electrodes for *in vivo* light delivery and recordings. A bilateral cannula was placed above the locus coeruleus (anteroposterior, –5.45mm; mediolateral, +/–1.0 mm; dorsoventral, 3.0 mm) for *in vivo* light delivery. An EEG/EMG implant was placed anterior to the cannula. EEG electrodes were placed on the skull above the frontal and parietal lobes, and EMG electrodes were placed within the neck musculature.



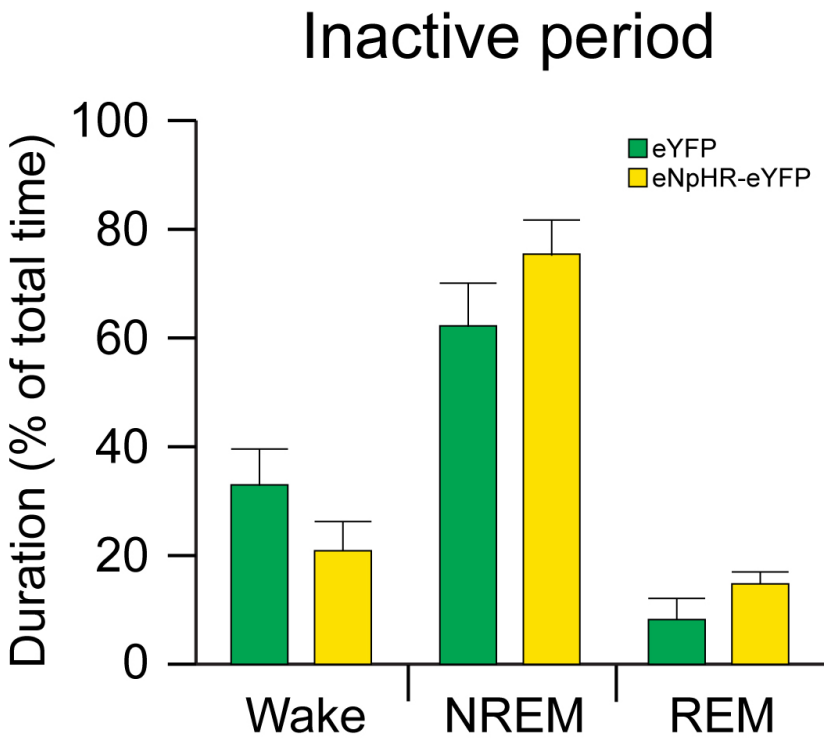
Supplementary Figure 4. Baseline sleep architecture of sham and virally-transduced animals**.** In both the light (inactive) and dark (active) periods, we found no significant difference in the percentage of wakefulness, NREM, or REM sleep between animals injected with saline or transduced with EF1α::eYFP, EF1α::eNpHR-eYFP, or EF1α::ChR2-eYFP (P>0.05, two-way ANOVA, n=4 animals).



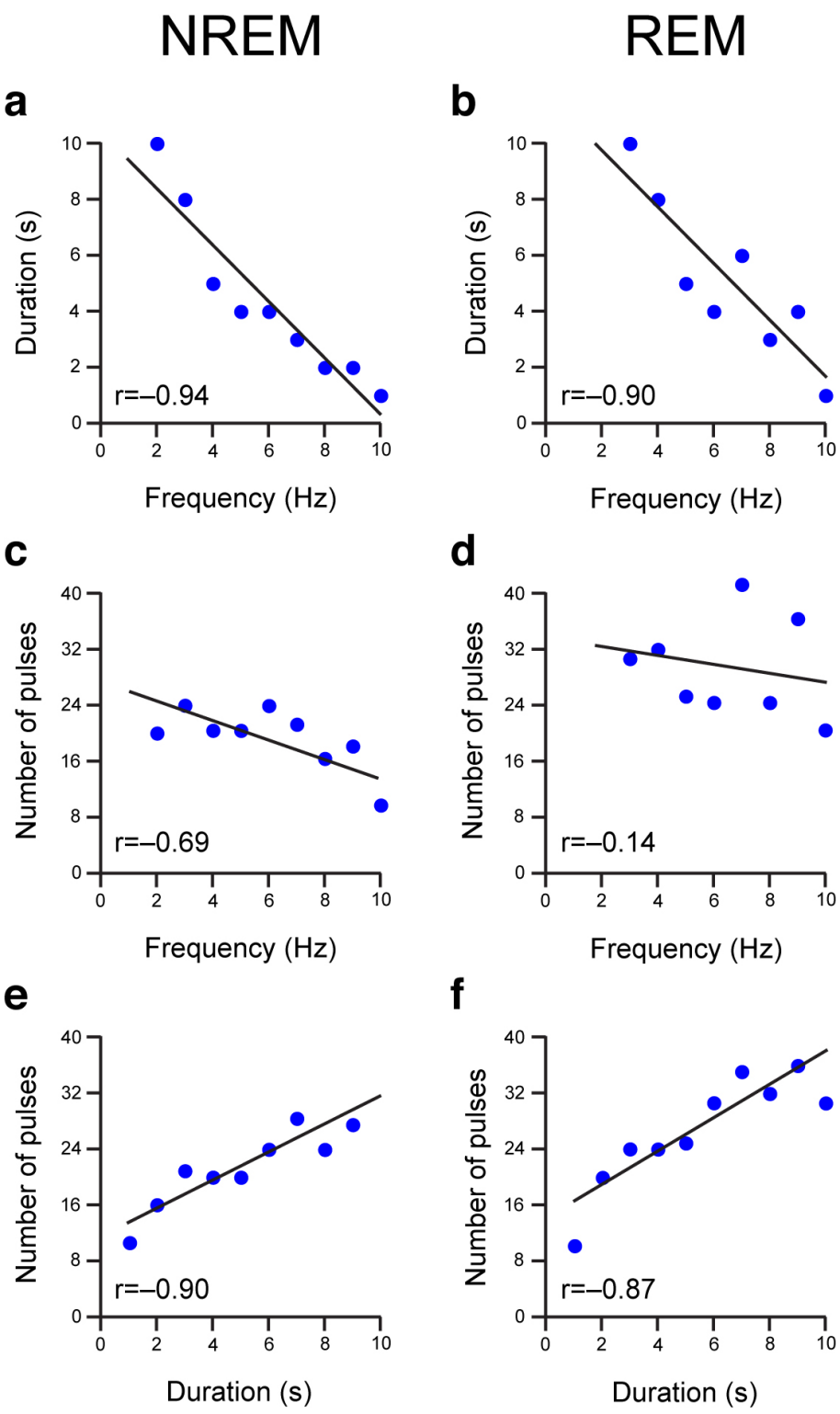
Supplementary Figure 5. *In vivo* photostimulation of locus coeruleus neurons increases c-Fos immunoreactivity in the locus coeruleus. **(a)** Representative images of the locus coeruleus co-stained for tyrosine hydroxylase (light brown) and c-Fos (black). Photographs depict histological samples from eYFP transduced mice (top row) and ChR2-eYFP transduced mice (bottom row) and represent the side of the brain that received unilateral stimulation (ipsilateral, left column) or the side of the brain that was not stimulated (contralateral, right column). **(b)** Quantification of the percentage of neurons showing tyrosine hydroxylase (TH) immunoreactivity that also express c-Fos. Data represent the mean +/– s.d. from eYFP (n=4) and ChR2-eYFP (n=4) animals. $^{**}P<0.001$, two-way ANOVA followed by Student's t-test. **(c)** Quantification of the number of neurons showing tyrosine hydroxylase immunoreactivity that also express c-Fos from adjacent 30 μm brain sections. Data represent the mean +/– s.d. number of neurons that express each label in the ipsilateral locus coeruleus in ChR2-eYFP animals (n=4).



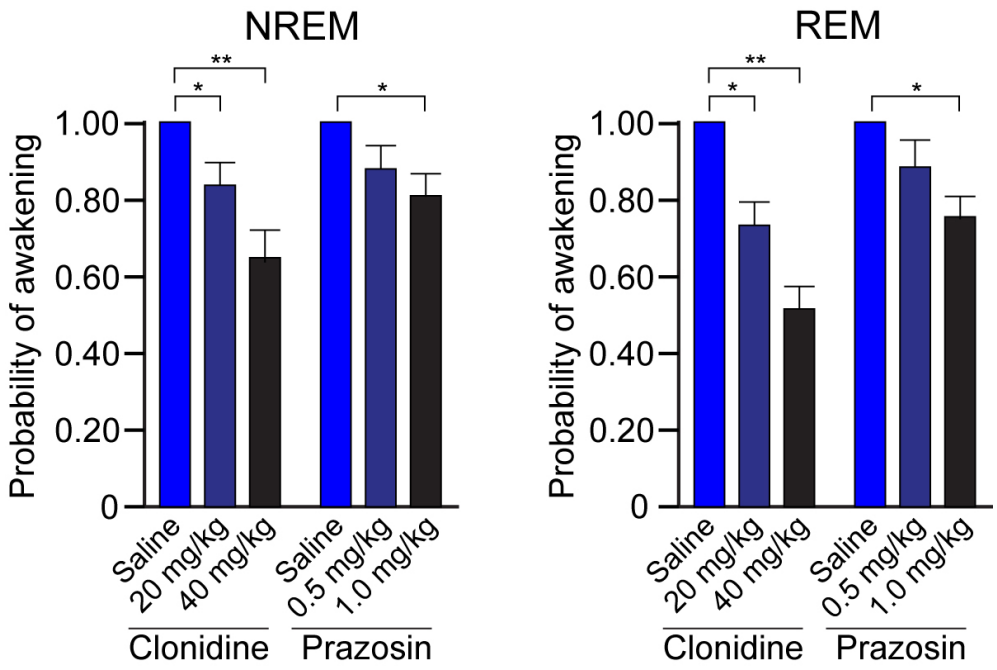
Supplementary Figure 6. *In vivo* photoinhibition of locus coeruleus neurons causes a decrease in norepinephrine content in prefrontal cortex. Data represent the mean +/– s.e.m. of 4 trials per animal, n=4 animals. ** $P<0.001$, two-way ANOVA between timepoint and virally-transduced animal followed by Bonferroni post-hoc test.



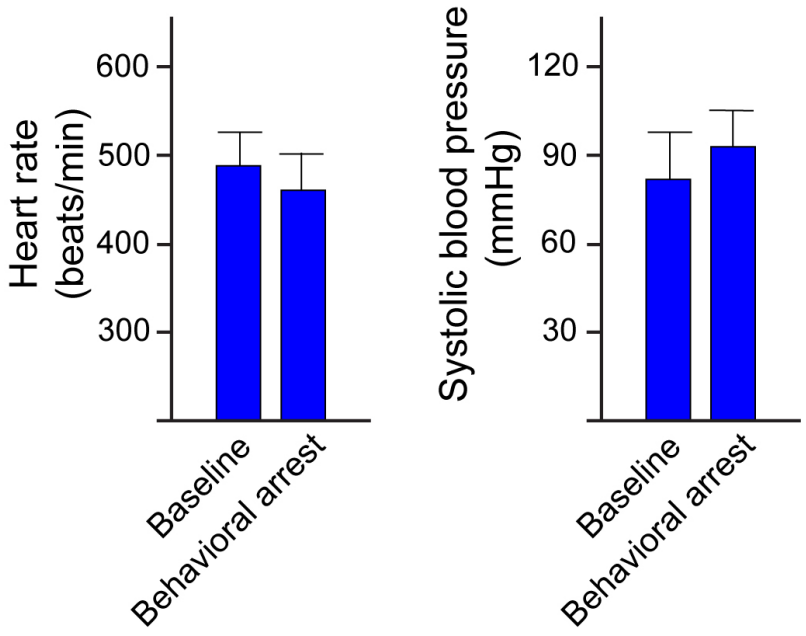
Supplementary Figure 7. The percentage of time spent in wake, NREM, and REM sleep during 1 h photoinhibition in the inactive (light) period. Data represent the mean +/– s.e.m. of 6 separate 1 h sessions, n=6 animals. **P*>0.05, two-tailed Student's t-test between transduced animals.



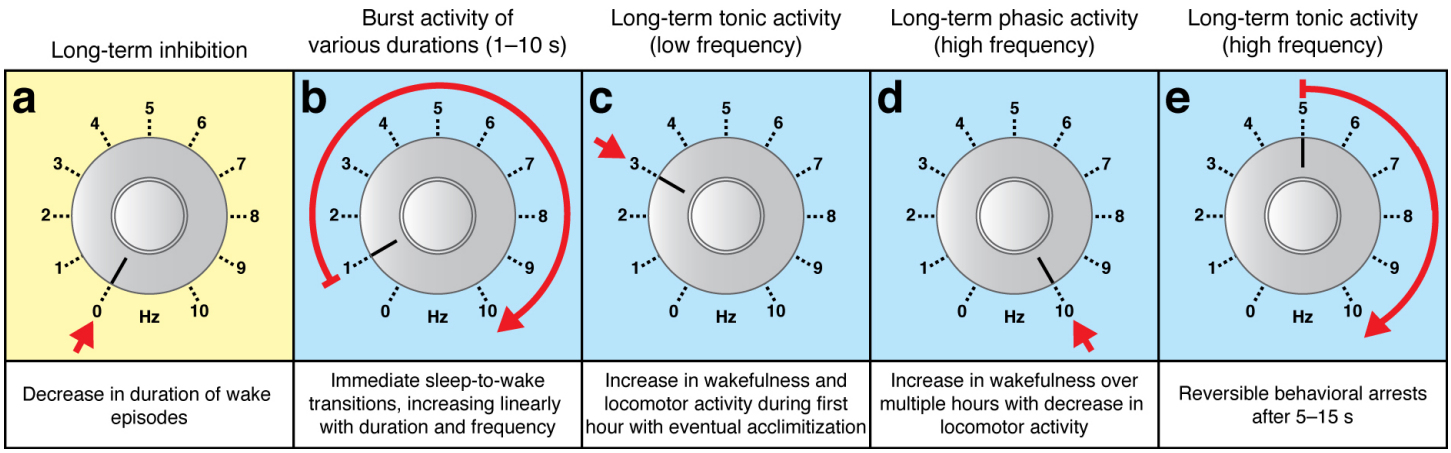
Supplementary Figure 8. Correlation between stimulation parameters and the probability of awakening from NREM (a,c,e) and REM (b,d,f) sleep. Each dot represents the lowest mean value across 6 stimulated ChR2-eYFP animals for which animals exhibited sleep-to-wake transitions in 100% of trials. **(a,b)** The relationship between the stimulation frequency (1–10 Hz) and duration (1–10 s) that caused a 100% probability of awakening. **(c,d)** The relationship between the number of pulses necessary to elicit a 100% probability of awakening and the stimulation frequency. **(e,f)** The relationship between the number of pulses necessary to elicit a 100% probability of awakening and the stimulation duration.



Supplementary Figure 9. The probability of a sleep-to-wake transition from NREM (left) or REM sleep (right) in the 10 s following the onset of photostimulation (10 ms pulses at 5 Hz for 5 s) in ChR2-eYFP transduced animals (n=4) following administration of an α2 receptor agonist (clonidine) or α1 receptor antagonist (prazosin). Data represent the mean +/– s.e.m after 12 trials per condition per mouse. Increased darkness of bars represents increased pharmacological dose. **P*<0.05, ***P*<0.001 two way ANOVA between saline and pharmacological conditions followed by Bonferroni posthoc test.



Supplementary Figure 10. Heart rate and systolic blood pressure before and during behavioral arrests. *P*>0.05, Student's t-test between epochs, 6 trials per animal in n=6 animals.



Supplementary Figure 11. Summary of the effects of manipulation of locus coeruleus activity on wakefulness. The locus coeruleus can be thought of as a tuning dial, with different frequencies causing different effects on behavior. Red arrows represent the specific frequencies tested in this study. **(a)** Photoinhibition with yellow light decreases the duration of wake-episodes. **(b)** Photostimulation with 10 ms pulses of blue light linearly increases the probability of a sleep-to-wake transition. **(c)** Long-term tonic stimulation with 10 ms pulses at 3 Hz increases wakefulness and locomotor activity over an hour, but the effects are lost over 5 h stimulation. **(d)** Phasic stimulation with 10 ms pulses at 10 Hz (lasting 500 ms every 20 s) increases wakefulness over both 1 and 5 hours, while locomotor activity decreases. **(e)** Phasic stimulation with 10 ms pulses at 5 Hz and above causes reversible behavioral arrests when applied for greater than 5–15 s.