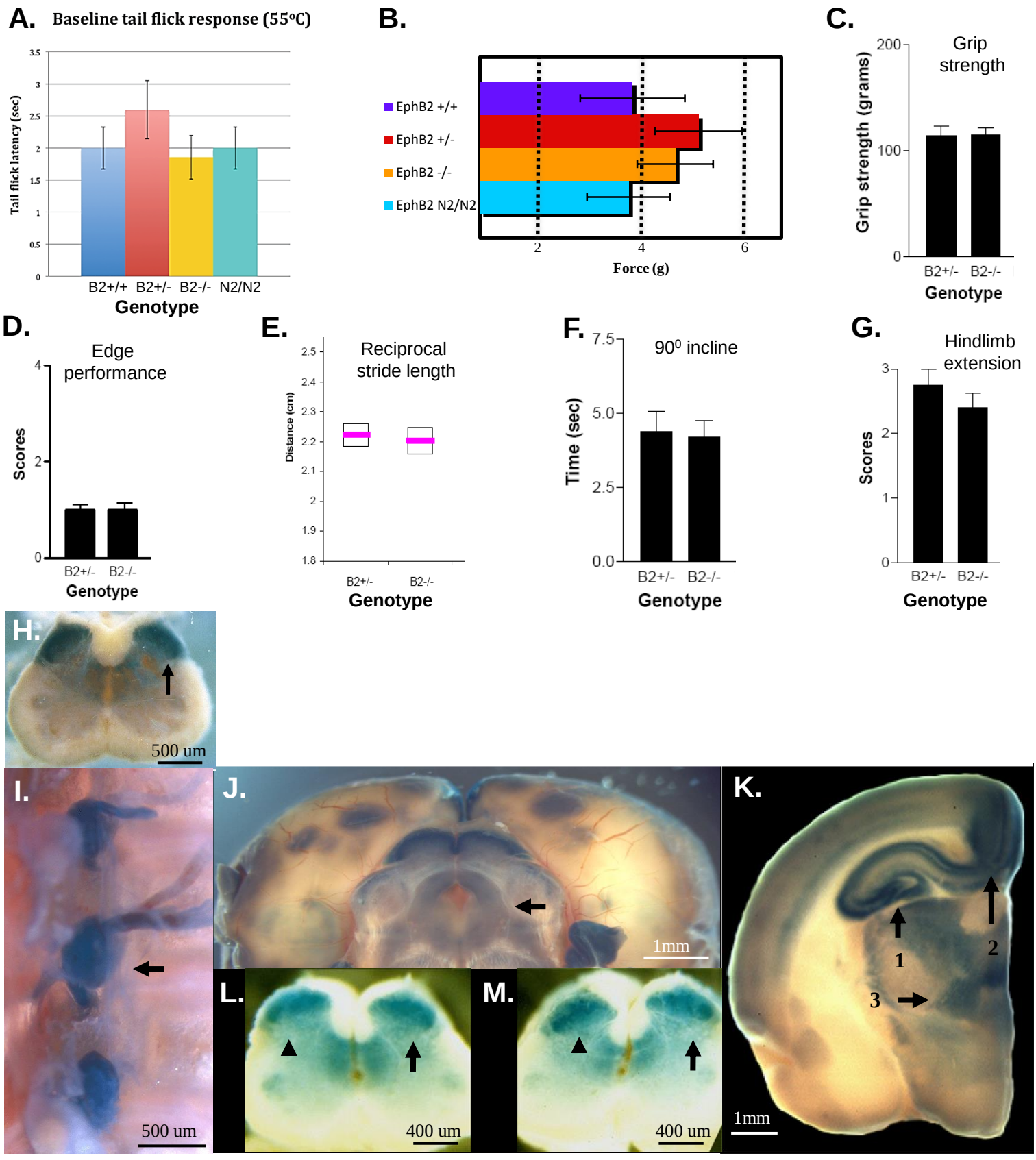
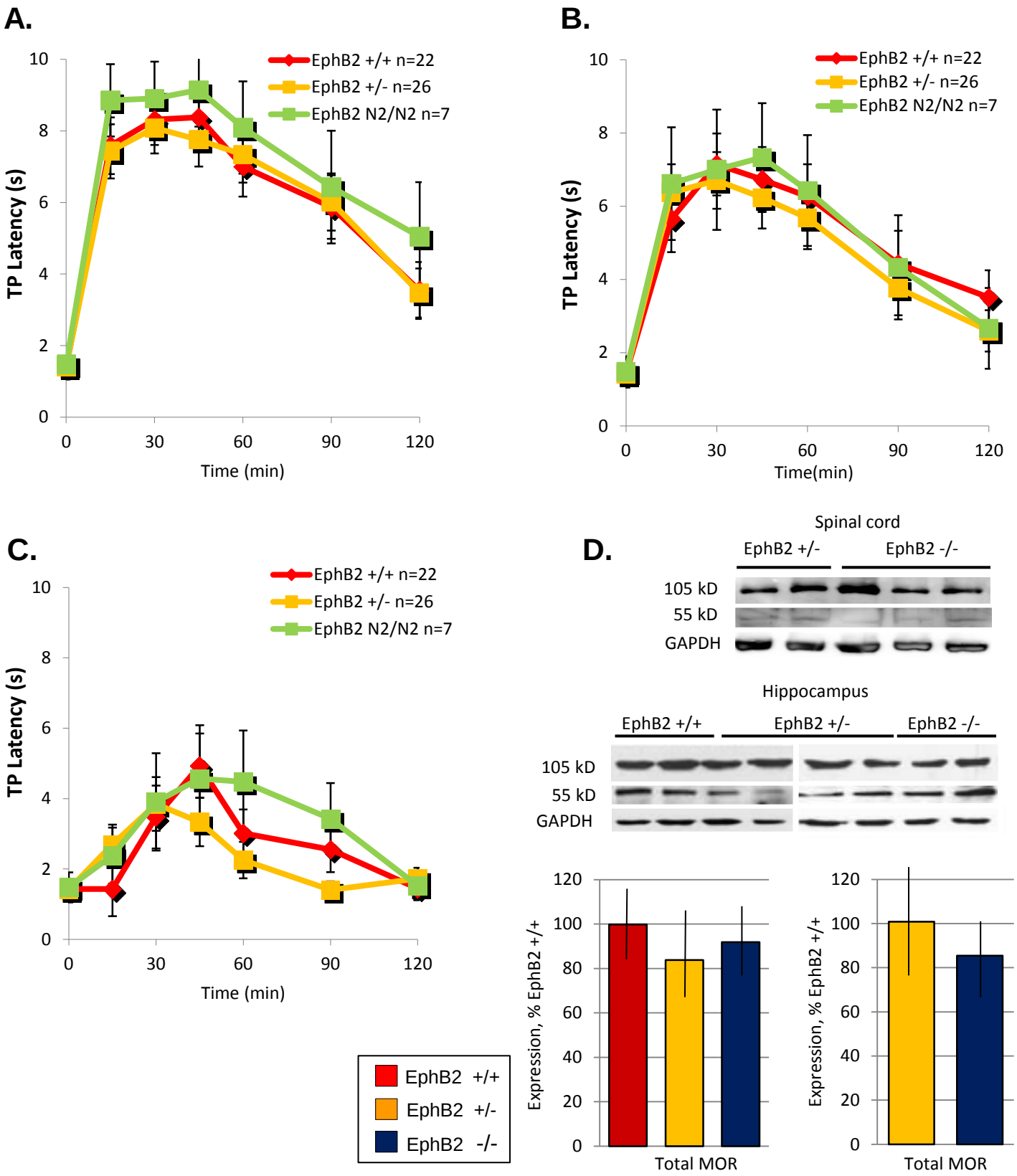
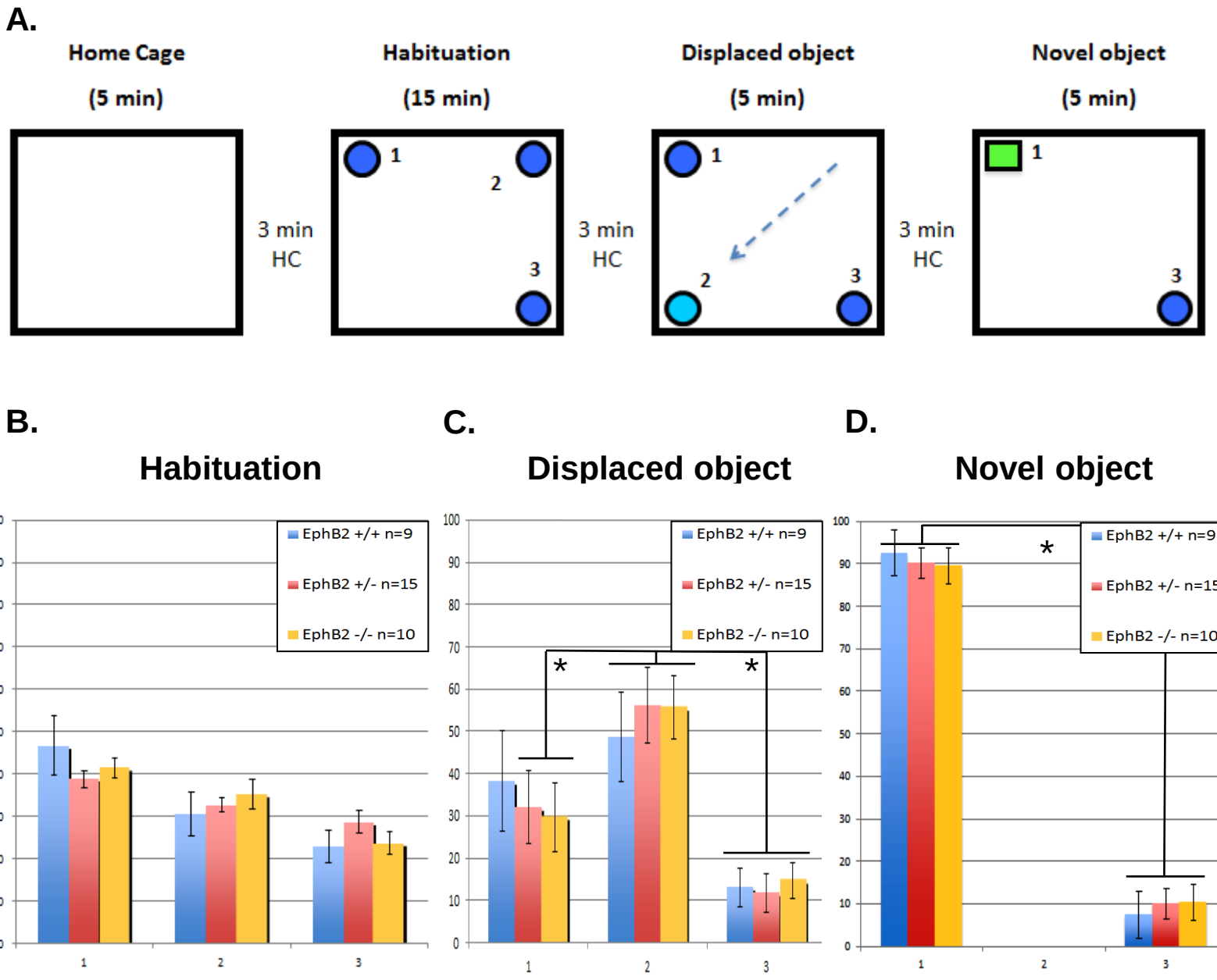


Supplementary Figure S1. (**A**) Baseline tail flick response at 55^{0}C, N= 13, 14, 12 and 8 for EphB2 +/+, +/-, -/- and N2/N2 mice respectively. (**B**) Relative von Frey plantar fibre response, N=8, 8, 8 and 5 for EphB2 +/+, +/-, -/- and N2/N2 respectively. (**C-G**) Measures of dynamic motor function in EphB2 heterozygous and null mice as indicated (see Methods), N=10 mice/genotype. (**H-M**) Expression of EphB2 in the adult CNS as demonstrated via beta-galactosidase histochemistry in EphB2 $^{N2/+}$ mice. (**H**) expression in dorsal lamina (I-III) of the lumbar spinal cord (L2), (**I**) Dorsal root ganglia in the lumbar spinal cord (L3-5), (**J**) expression in periaqueductal gray (arrow), (**K**) 1-hippocampus 2- anterior cingulate cortex, 3-thalamic nuclei. Examples of EphB2 expression in dorsal spinal lamina: ipsilateral (arrow) or contralateral (arrowhead) one week following either sciatic nerve transection (**L**) or chronic constriction injury (**M**). Error bars are ±SEM.



Supplementary Figure S2. Analysis of antinociceptive responses to tail pinch following morphine administration in wild type (red diamond), heterozygous (yellow square) and EphB2 N2/N2 mice (green square) (**A**) Antinociceptive responses following initial exposure to morphine on day 1. No significant difference in tail pinch latencies between all 3 animal groups. (**B**) Tail pinch latencies on day 3. No significant difference in tail pinch latencies between all 3 animal groups. (**C**) Tail pinch latencies on day 6. No significant difference in tail pinch latencies between all 3 animal groups. (**D**) Mu opioid receptor levels as function of EphB2 wild type (spinal levels L1-L4 and hippocampus). No significant difference in total MOR levels were observed between EphB2 +/+, +/- or -/- mice ($n \leq 4$ animals/group). Error bars are shown ± SEM, *= $p \leq 0.05$, TP= tail pinch.



Supplementary Figure S3. Novel object recognition in EphB2 mice. (**A**) Schematic of procedural operations performed. Mice were placed in an empty clear plastic test cage in the presence of visual cues for 5 minutes, followed by a habituation phase with the initial test objects for an additional 15 minutes. Object 2 was then displaced to the novel position shown and interactions assessed for a period of 5 minutes. A new novel object was then introduced into the test cage and animal interrogations examined over an additional 5 minute period. (**B**) Soring of object interaction by genotype, habituation phase, (**C**) Soring of object interaction by genotype, displaced object (**D**) Soring of object interaction by genotype, novel object recognition. Note that EphB2 null mice exhibit no significant differences in either basal habituation, object displacement, or novel object recognition tasks compared to littermate controls. For all tasks, N≤9 animals/group). Error bars are shown ± SEM, *= p≤0.05, TP= tail pinch.