SUPPLEMENTAL MATERIALS

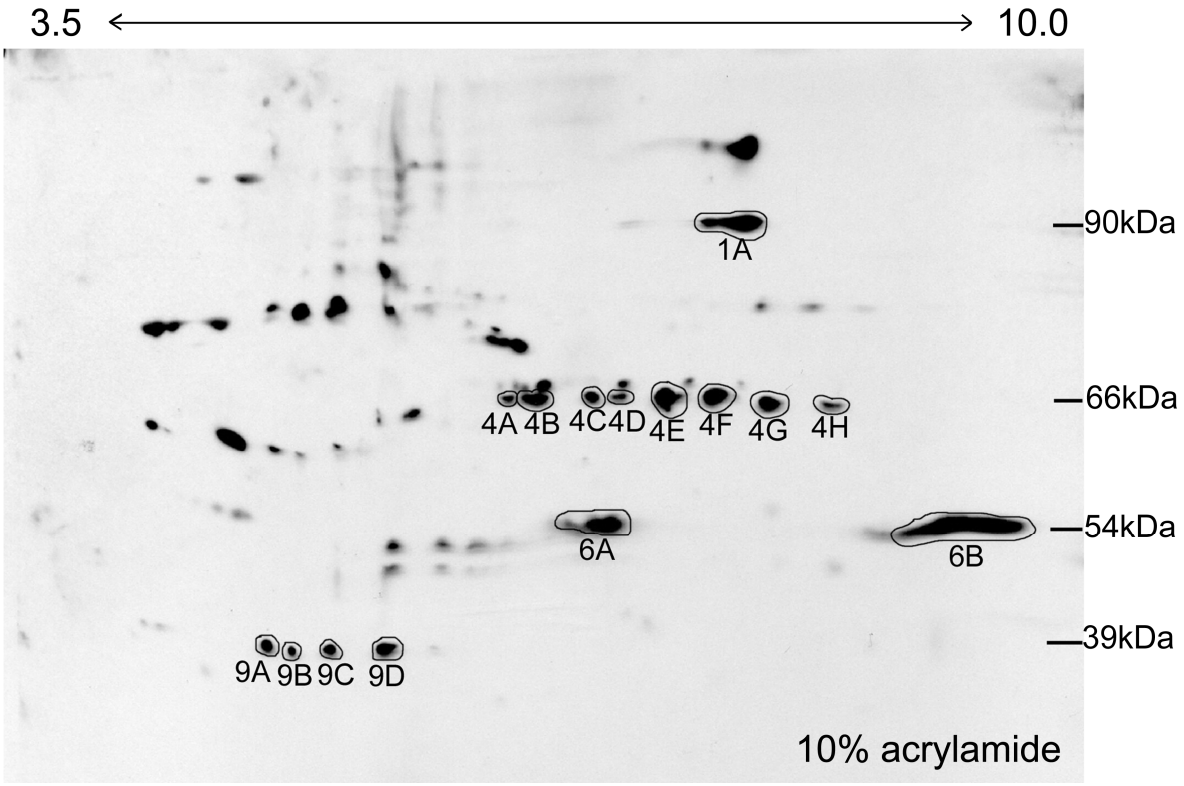
METHODS

Primary cell culture

NAc cells were obtained from postnatal day 1 rats and primary cultures prepared as described previously (Mangiavacchi & Wolf, 2004). After 2-3 weeks in vitro, cells were fixed (4% paraformaldehyde, 15 min), permeabilized (0.1% Triton X-100, 15 min), blocked with 5% donkey serum (60 min), incubated with antibody to either CRMP-2 (1:100, IBL America) or $G_{\alpha o}$ (1:200; Santa Cruz Biotechnology) for 1 h at RT, and then incubated with Alexa-488 donkey anti-mouse secondary antibody (1:1000; Molecular Probes) to visualize CRMP-2 or a Cy3 donkey anti-rabbit secondary antibody (1:1000; Jackson Labs) to visualize $G_{\alpha o}$. Images were acquired with a Nikon inverted microscope, an ORCA-ER digital camera and MetaMorph software (Universal Imaging, Downingtown, PA).

FIGURES

Supplemental Figure 1. Western blot of a 2-dimensional gel immunoprobed with antibody recognizing phosphorylated PKA substrates. **1A,** PKA substrate of 90kDa; **4A-H,** PKA substrate of 66kDa; **6A-B,** PKA substrate of 54kDa; **9A-D,** PKA substrate of 39kDa. The first dimension of isoelectric focusing [pI] is indicated along the top, and the second dimension of separating by mass is indicated by molecular weight standards shown on the right.



Supplemental Figure 2. Representative images of medium spiny neurons in primary cultures prepared from the postnatal rat NAc. Cultures were fixed prior to incubation with primary antibody and fluorescent secondary antibody. Both CRMP-2 (panel **A**) and $G_{o\alpha 2}$ (panel **B**) are expressed throughout the processes and in cell bodies of medium spiny neurons.