<u>Supplemental Material</u>

Acquisition and performance of goal-directed instrumental actions depends on ERK signaling in distinct regions of dorsal striatum in rats

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Supplemental Table 1: tERK odu values in the DMS and DLS following instrumental training

Region	Group	isoform	Mean todu	SEM
DMS	CRF	tERK1	16168	3523
		tERK2	270732	29624
	Yoked	tERK1	15608	1052
		tERK2	245838	42081
	ACQ	tERK1	24328	5779
		tERK2	254920	21236
	ADD	tERK1	19071	1814
		tERK2	219044	18832
DLS	CRF	tERK1	35402	4719
		tERK2	202439	39229
	Yoked	tERK1	32801	6154
		tERK2	147503	36652
	ACQ	tERK1	36063	11132
		tERK2	228696	12985
	ADD	tERK1	31759	18118
		tERK2	234946	10619

tERK odu values from western blot analysis. Values are means ± 1 SEM. No effect of group or region was observed on tERK1 or tERK2 odu values ($p > 0.05$).

Supplemental Methods:

Immunohistochemistry: Rats were given an overdose of pentobarbital and perfused transcardially with 0.1 m phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Brains were post-fixed and cryoprotected in a solution of 20% sucrose in 4% PFA for 12-24 hours then frozen until sectioning. 40 um sections were cut and immersed in ice cold PBS, exposed to 30% peroxide solution in PBS, blocked with avidin and incubated with the primary

antibody diluted in PBS with 1% triton X-100 for 24-48 hours at 4 °C. After PBS washes the sections were incubated with a biotinylated secondary antibody for 2 hours at room temperature after which antibody binding was visualized using the Vector ABC kit (Vector Laboratories, Burlingame CA).

Quantification of p-ERK immunoreactivity: A particle density analysis of digital images (NIH ImageJ) was used to quantify p-ERK immunoreactivity.   ROI's were selected for DLS (Figure S1 Region 1) and the pDMS (Figure S1 Region 2) and the number of particles (designated as clusters of 5 or greater pixels that exceeded the threshold density) within each ROI were counted with the ImageJ particle analysis tool.To account for differences in background staining, image thresholds were adjusted prior to counting to equate particle density counts in a posterior portion of the dorsal striatum (Figure S1 Region 3).
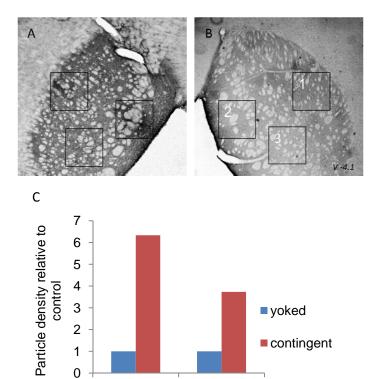

Supplemental Figure legend

Figure S1: Instrumental action-outcome learning increases ERK activation in the pDMS and DLS. Horizontal sections were taken from an instrumentally trained animal **(A)** and a yoked control animal **(B)** and stained for p-ERK. Quantification of p-ERK staining was performed in ROI's centered on the DLS (Region 1) and pDMS (Region 2). To equate background staining, prior to counting the image thresholds were equated for a third region (Region 3). **(C)** Training with contingent rewards resulted in an approximately 6-fold increase in particle density in the pDMS and 3-fold increase in the DLS compared to the yoked control animal.

Supplemental Figure S1: