

Withania extract operates through M1 receptor

M1 muscarinic receptor is a key target of neuroprotection, neuroregeneration and memory recovery by i-Extract from *Withania somnifera*.

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Supplementary Tables 1-6

Crude gels from Fig. 4

Crude gels from Fig. 5

Supplementary Table 1. Percent changes in KLK8 qRT-PCR and WB from saline or scopolamine-treated mice.

Treatment	KLK8 <i>in situ</i> RNA				KLK8 qRT-PCR				KLK8 WB				MAP2c WB			
	Change from SA		Change from SC		Change from SA		Change from SC		Change from SA		Change from SC		Change from SA		Change from SC	
	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc
SA	+0%	+0%	+24%	§+285%	+0%	+0%	§+38%	§+251%	+0%	+0%	§+94%	§+300%	+0%	+0%	*+60%	*+188%
SC	-19%	*-74%	+0%	+0%	*-27%	*-71%	+0%	+0%	*-48%	*-75%	+0%	+0%	*-37%	*-65%	+0%	+0%
SC → i-E	+5%	+8%	+30%	§+317%	*+35%	-7%	§+86%	§+228%	+33%	-32%	§+159%	§+174%	*+21%	*-24%	*+93%	*+118%
i-E	*+57%	*+132%	§+95%	§+794%	*+120%	*+99%	§+204%	§+597%	*+78%	*+200%	§+245%	§+1100%	*+65%	*+44%	*+164%	*+314%
i-E → SC	*+30%	*+40%	§+61%	§+441%	*+42%	*+31%	§+96%	§+360%	+39%	*+134%	§+170%	§+837%	*+15%	+11%	*+83%	*+220%

*Difference significant from SA at $p \leq 0.05$.

§Difference significant from SC at $p \leq 0.05$.

Supplementary Table 2 ANODE of mouse brain treatments vs. regions

Term	KLK8 in situ RNA		KLK8 qRT-PCR		KLK8 WB		MAP2c WB	
	χ^2 (df)	p	χ^2 (df)	p	χ^2 (df)	p	χ^2 (df)	p
Treatment	1045.2 (4)	<0.001	571.5 (4)	<0.001	651.2 (4)	<0.001	633.5 (4)	<0.001
Region	822.8 (1)	<0.001	0.1 (1)	0.715	299.2 (1)	<0.001	33.4 (1)	<0.001
Treatment \times Region	1004.8 (4)	<0.001	162.0 (4)	<0.001	554.6 (4)	<0.001	245.6 (4)	<0.001

Supplementary Table 3. Percent changes in KLK8 qRT-PCR and WB from saline or scopolamine-treated mice vs M1 receptor agonist or antagonist treatment.

Treatment	KLK8 qRT-PCR				KLK8 Western				MAP2c Western			
	Change from SA		Change from SC		Change from SA		Change from SC		Change from SA		Change from SC	
	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc	Cortex	Hippoc
SA	+0%	+0%	§+172%	§+775%	+0%	+0%	§+315%	§+877%	+0%	+0%	§+221%	§+195%
M1 ant	*-61%	*-74%	+6%	§+127%	*-70%	*-70%	§+26%	§+196%	-67%	*-72%	+5%	-18%
M1 ag	*+157%	*+146%	§+601%	§+2055%	*+152%	*+148%	§+946%	§+2324%	*+160%	*+115%	§+734%	§+532%
SC	*-63%	*-89%	+0%	+0%	*-76%	*-90%	+0%	+0%	-69%	*-66%	+0%	+0%
M1 ag → SC	*+121%	*+92%	§+501%	§+1582%	*+113%	*+94%	§+786%	§+1796%	*+120%	+23%	§+606%	§+263%
i-E → SC	*+87%	*+99%	§+409%	§+1644%	*+89%	*+101%	§+686%	§+1862%	*+86%	*+51%	§+498%	§+344%
M1 ant → i-E → SC	+0%	*-69%	§+173%	§+174%	+7%	*-64%	§+343%	§+247%	+3%	*-73%	§+230%	-20%
i-E	*+223%	*+171%	§+780%	§+2272%	*+220%	*+171%	§+1230%	§+2547%	*+227%	*+163%	§+950%	§+674%
M1 ant → SC	-9%	*-74%	§+148%	§+124%	-13%	*-73%	§+260%	§+165%	-17%	*-84%	§+166%	-53%

*Difference significant from SA at $p \leq 0.05$.

§Difference significant from SC at $p \leq 0.05$.

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Supplementary Table 4 ANOVA of mouse brain treatments, including M1 agonist and antagonist, vs. brain regions

Term	KLK8 qRT-PCR		KLK8 WB		MAP2c WB	
	χ^2 (df)	p	χ^2 (df)	p	χ^2 (df)	p
Treatment	1627 (8)	<0.001	1845 (8)	<0.001	267 (8)	<0.001
Region	50 (1)	<0.001	55 (1)	<0.001	169 (1)	<0.001
Treatment \times Region	410 (8)	<0.001	445 (8)	<0.001	288 (8)	<0.001

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Supplementary Table 5. Gavage and Injection Contents for data in Fig. 4

Treatment	Control	Scop.	Scop. → i-Extract	i-Extract	i-Extract → Scop.
Gavage	0.5% DMSO	0.5% DMSO	i-Extract in 0.5% DMSO	i-Extract in 0.5% DMSO	i-Extract in 0.5% DMSO
Injection	0.9% saline	Scop. In 0.9% Saline	Scop. in 0.9% saline	0.9% saline	Scop. in 0.9% saline
Order of drugs and separation time	0.9% saline 1h↓	Scop in 0.9% saline 1h ↓	Scop in 0.9% saline 1h ↓	0.9% saline 1h↓	i-Extract in 0.5% DMSO 1h↓
	0.5%DMSO	0.5%DMSO	i-Extract in 0.5% DMSO	i-Extract in 0.5% DMSO	Scop in 0.9% saline

Table lists contents of gavage and injection treatments given to animal groups as portrayed in Fig. 2. Note that multiple substances were not given simultaneously but were separated by hours as specifically described in main text.

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Supplementary Table 6. Gavage and Injection Contents for data in Fig. 5

Treatment	Control	Antagonist	Agonist	Scop.	Agonist → Scop.	i-E → Scop.	Antagonist → i-Extract → Scop.	i-Extract	Antagonist → i-Extract
Gavage	0.5% DMSO	0.5% DMSO	0.5% DMSO	0.5% DMSO	0.5% DMSO	i-Extract in 0.5% DMSO	i-Extract in 0.5% DMSO	i-Extract in 0.5% DMSO	i-Extract in 0.5% DMSO
Injection	saline	Antagonist in saline	Agonist in saline	Scop. in Saline	Agonist + Scop. In saline	Scop. in saline	Antagonist + Scop. in saline	Saline	Antagonist in Saline
Order of drugs and separation time	0.9% saline 1h ↓ 0.5% DMSO	Antagonist in 0.9% saline 1h ↓ 0.5% DMSO	Agonist in 0.9% saline 1h ↓ 0.5% DMSO	Scop. in 0.9% saline 1h ↓ 0.5% DMSO	Agonist in 0.9% saline 2h ↓ Scop. in 0.9% saline 1h ↓ 0.5% DMSO	i-Extract in 0.5% DMSO 1h ↓ Scop. in 0.9% saline	Ant in saline 1h ↓ i-Extract in 6% DMSO 1h ↓ Scop. in saline	0.9% saline 1h ↓ i-Extract in 0.5% DMSO	Ant in saline 2h ↓ i-Extract in 6% DMSO

Table lists contents of gavage and injection treatments given to animal groups as portrayed in Fig. 3. Note that multiple substances were not given simultaneously but were separated by hours as specifically described in main text.

Fig. 4B gels

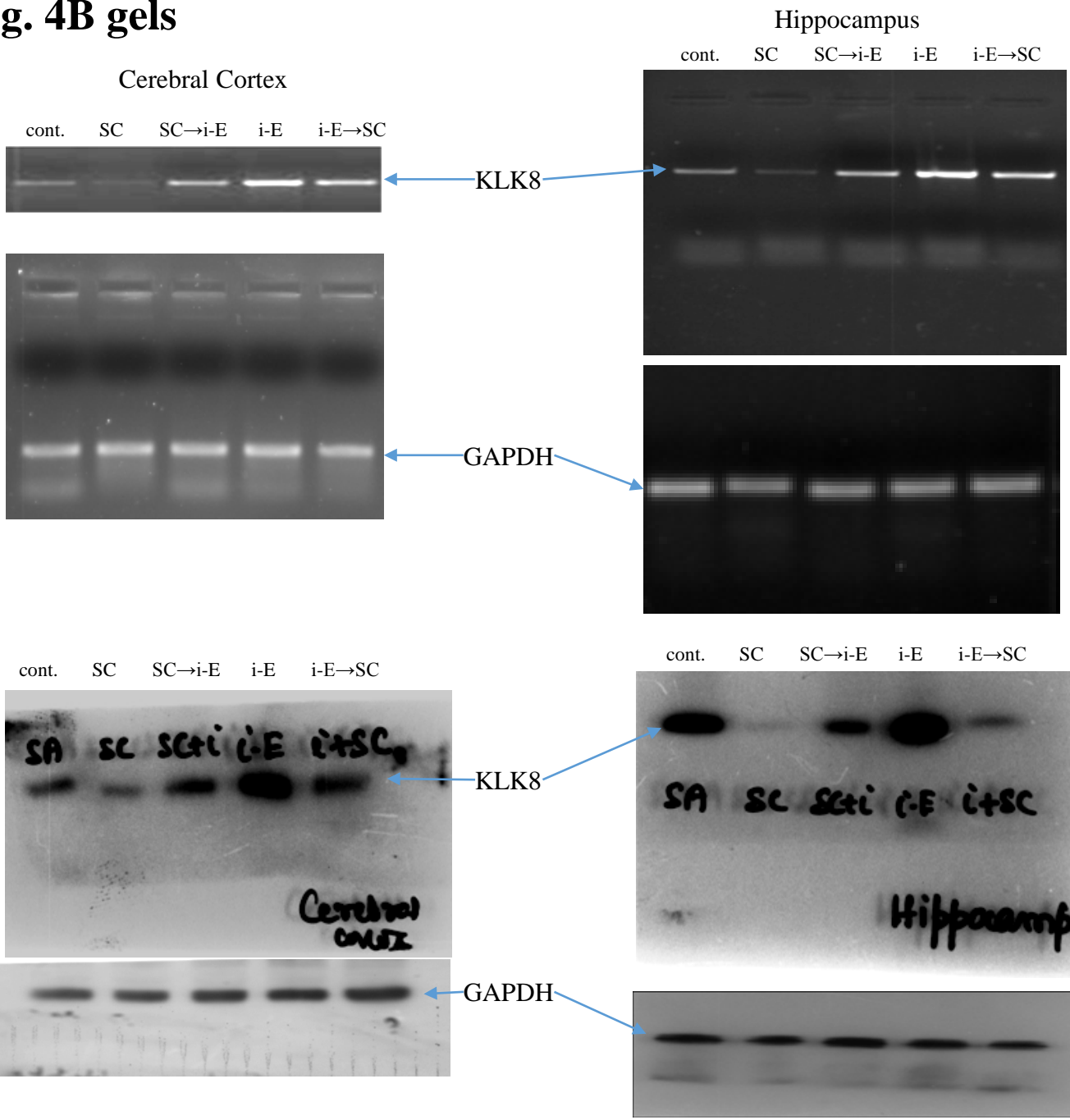


Fig. 4C gels

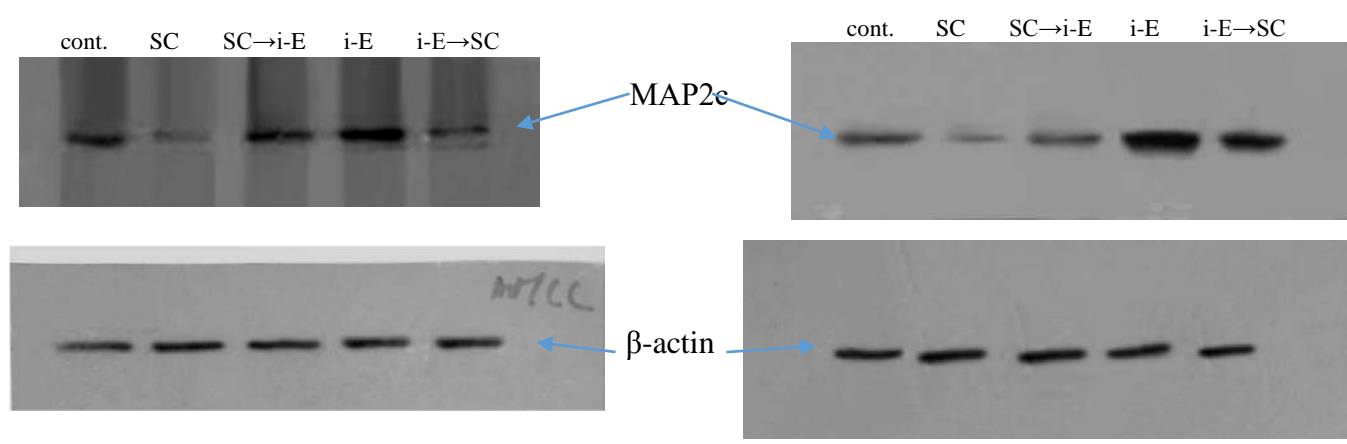


Fig. 5A gels

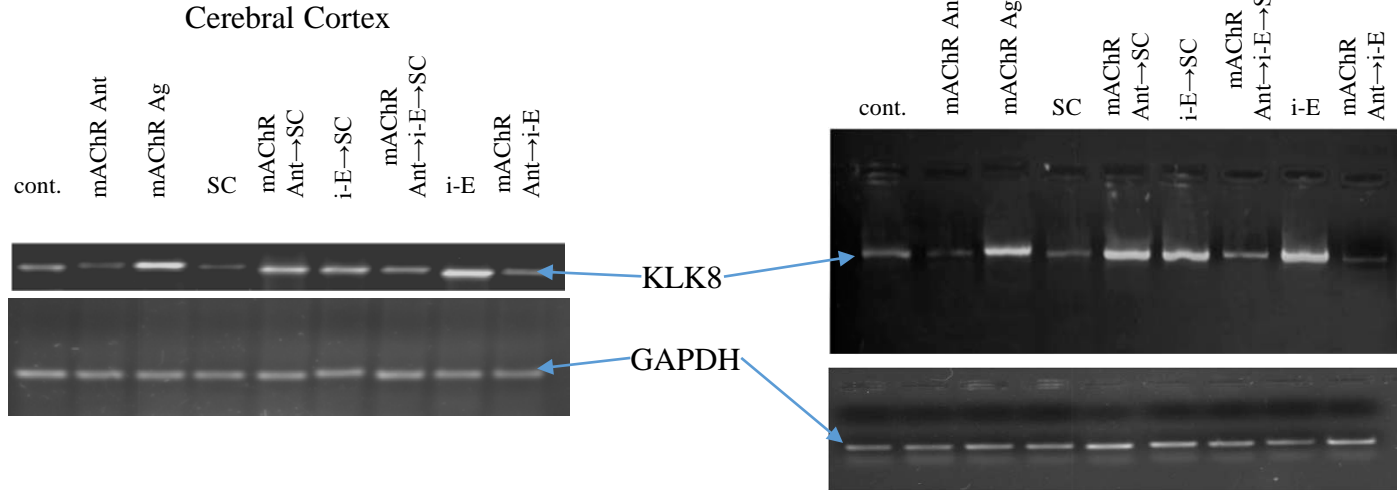


Fig. 5B gels

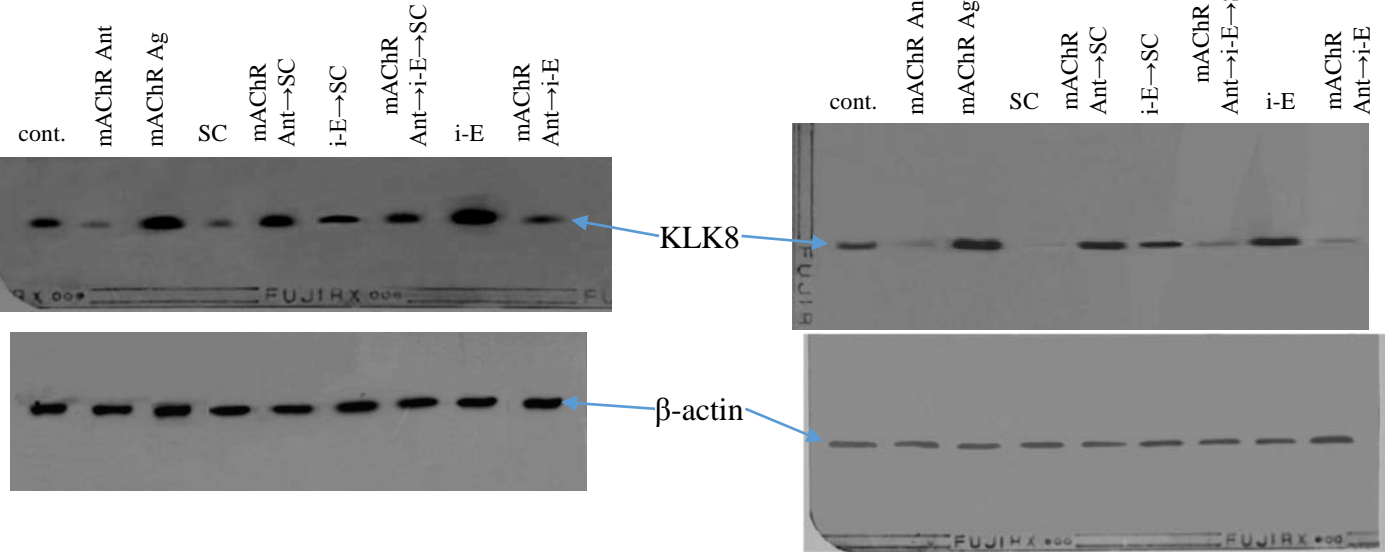


Fig. 5C gels

