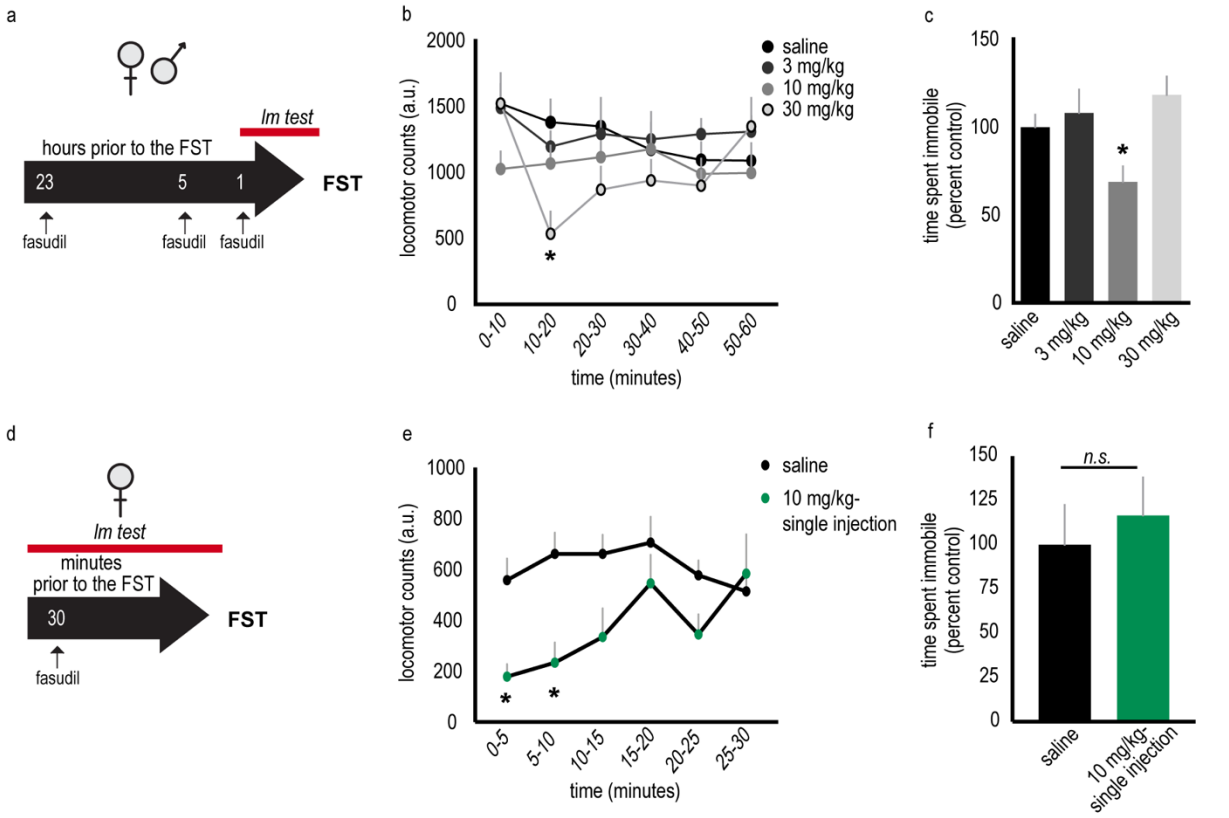
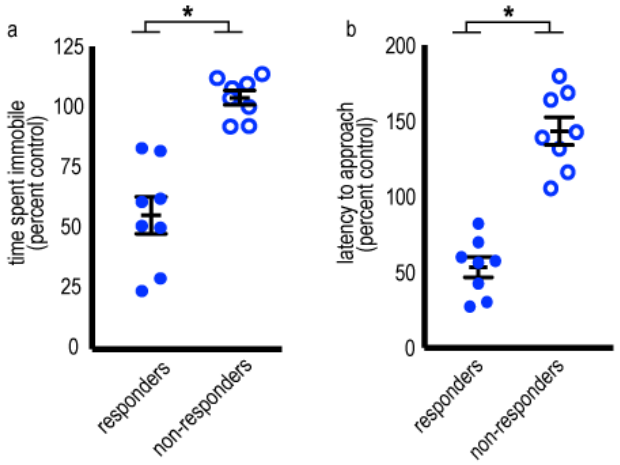
Supplementary materials, Shapiro et al.

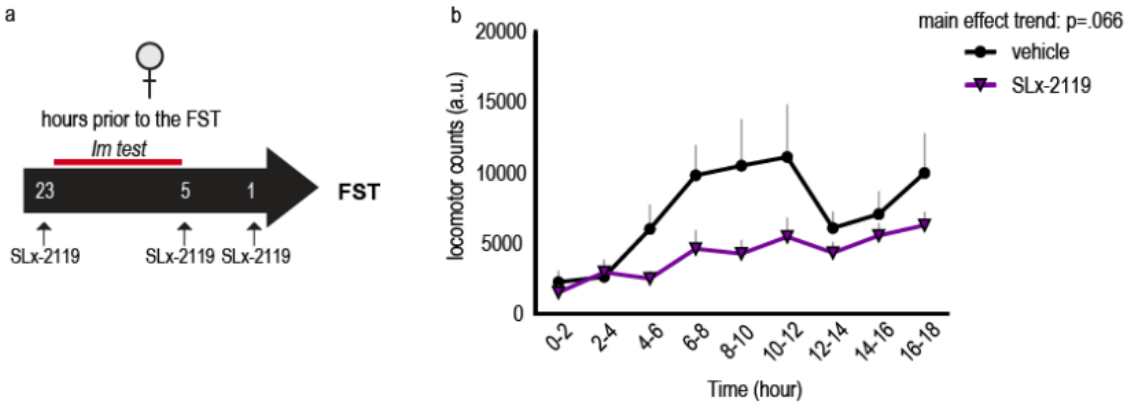
Supplementary figures



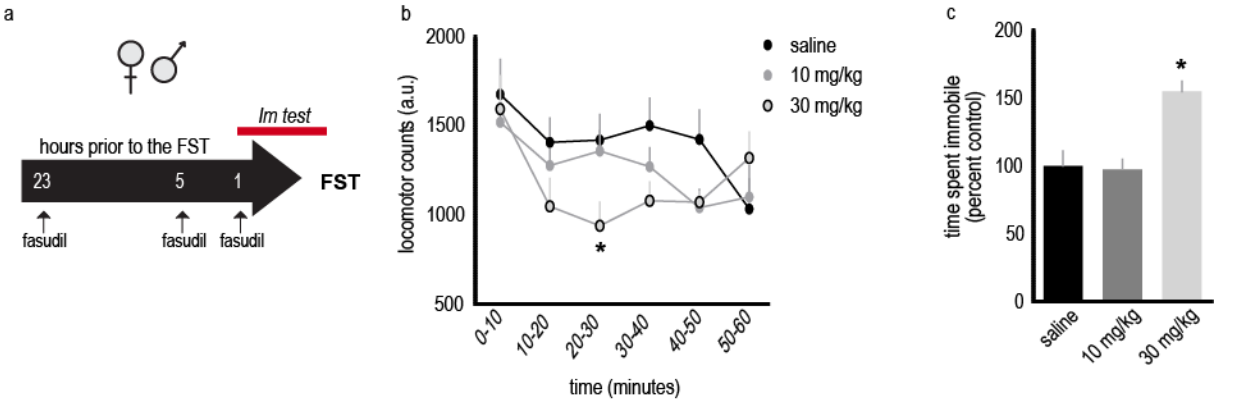
Supplementary figure 1. Differential behavioral effects of single *vs.* multiple injections and doses of fasudil. A. Fasudil was administered to adolescent male and female mice 23, 5 and 1 hour prior to the forced swim test (FST). Between the final injection and FST, mice were placed in locomotor monitoring chambers. “lm test” refers to locomotor test. **B.** 30 mg/kg fasudil transiently decreased locomotor activity, but groups did not differ by the start of the FST at 60 minutes [interaction $F_{(15,130)}$=2.55, p=0.002], n=7-8/group. **C.** As reported in the main text, 10 mg/kg fasudil reduced the time spent immobile in the FST, an antidepressant-like effect [$F_{(3,56)}$=4.91, p=0.004], n=7-24/group. **D.** A single injection of 10 mg/kg fasudil was administered to adolescent female mice immediately before the start of a locomotor test, and 30 minutes before the FST. **E.** Fasudil briefly reduced locomotion, however groups did not differ by the start of the FST at 30 minutes [interaction $F_{(5,30)}$=2.57, p=0.047], n=4/group. **F.** A single injection of fasudil 30 minutes before the FST did not alter time spent immobile [$t_{(14)}$=0.53,p=0.60], n=8/group. Means + SEMs, *p<0.05.



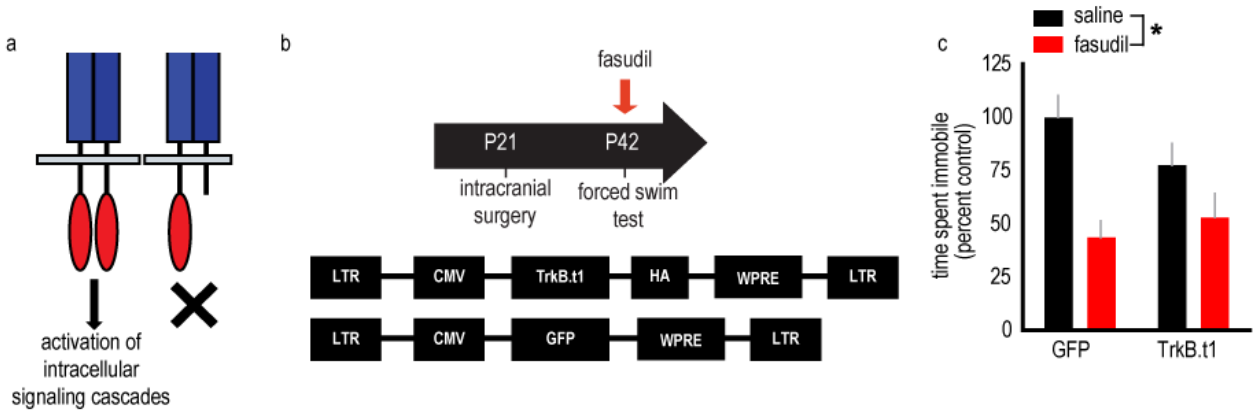
Supplementary figure 2. Ketamine has variable behavioral effects in adolescent mice. A median split was used to identify ketamine "responders" and "non-responders" in **(A)** the forced swim test ($t_{(14)}$=5.94, p<0.0001) and **(B)** in separate mice, the novelty suppressed feeding test ($t_{(14)}$=7.94, p<0.0001), n=8/group. Individual mice are plotted, with means ± SEMs in black lines, *p<0.05.



Supplementary figure 3. Effects of SLx-2219 on locomotor activity. A. SLx-2119 was administered to adolescent female mice 23, 5 and 1 hour prior to the forced swim test (FST), as indicated by the arrows. Mice were placed into locomotor monitoring chambers for an 18-hour period as indicated. "lm test" refers to locomotor test. **B.** We identified a trend towards blunted activity [$F_{(1,16)}$=3.91, p=0.066], n=8-10/group. Means + SEMs.



Supplementary figure 4. Fasudil does not have antidepressant-like efficacy in adult mice. A. Fasudil was administered to adult male and female mice 23, 5 and 1 hour prior to the forced swim test (FST). Between the final injection and FST, mice were placed in locomotor monitoring chambers. "lm test" refers to locomotor test. **B.** The 30 mg/kg dose briefly blunted locomotor activity [interaction $F_{(10,105)}$=2.81, p=0.004], but groups did not differ by the start of the FST at 60 minutes. **C.** In adults, neither dose tested elicited antidepressant-like effects in the FST. Instead, 30 mg/kg increased time spent immobile [$F_{(2,22)}$=9.78, p=0.0009], n=7-11/group. Means + SEMs, *$p \leq 0.05$.



Supplementary figure 5. Overexpression of *TrkB.t1* does not alter the antidepressant-like effects of fasudil in the forced swim test (FST). A. Upon ligand binding, TrkB dimerizes. There are two isoforms of TrkB: full-length and truncated. The full-length isoform contains a kinase domain that autophosphorylates to activate intracellular signaling cascades, whereas the truncated isoform lacks this domain and thus cannot activate canonical intracellular signaling cascades. **B.** Experimental timeline: at postnatal day (P) 21, a lentivirus expressing truncated TrkB (TrkB.t1) or green fluorescent protein (GFP) (bottom; see also (Rattiner et al 2004)) was infused into the ventromedial prefrontal cortex. Viral vector spread was consistent with that shown in main text, fig. 5c. At P42, mice were tested in the FST. **C.** Fasudil decreased the time spent immobile, regardless of viral vector group, suggesting that the antidepressant-like efficacy of

fasudil in this test is not dependent upon its modification of TrkB.t1 (reported in the main text) [main effect of fasudil $F_{(1,20)}$=15.68, p<0.001; no effect of virus $F_{(1,20)}$=0.41, p=0.529; no interactions], n=5-7/group. Means + SEMs, *p<0.05.

Supplementary methods associated with supplementary figure 5

TrkB.t1- or green fluorescent protein (GFP)-expressing constructs were packaged into lentiviruses with a CMV promoter and HA tag by the Emory University Viral Vector Core. This viral vector has been previously described (Rattiner et al 2004) and validated in the PFC (Pitts et al 2018). Surgical and behavioral testing procedures were as described in the main text.

Following euthanasia, the viral vectors were visualized by imaging the GFP tag or immunostaining for HA, as appropriate. Paraformaldehyde-fixed tissue was blocked with 2% normal goat serum and 1% BSA in 1X PBS + 0.4% triton X-100 and incubated in an anti-HA antibody (anti-rabbit, Sigma H6908, lot #05m4801v, 1:1000) overnight at 4ºC. Sections were then washed in 1X PBS and incubated in an Alexa Fluor 488 goat anti-rabbit secondary antibody (Jackson ImmunoResearch 111-545-144, lot #121665, 1:500) for 1 hour at room temperature and were mounted and imaged. Viral vector spread was consistent with main text fig. 5c. In addition, some ventral spread along the corpus callosum was noted in some animals, but we have no evidence that this spread impacted our findings. Mice with infusions that were unintentionally rostral, infecting the orbitofrontal cortex, were excluded.

Supplementary tables

Supplementary table 1.

	Scramble	ROCK2 shRNA
n (cells)	49	52
I_H ratio	0.062±0.003	0.063±0.004
$I_{K(IR)}$ ratio	2.21±0.08	2.18±0.06
Tau (ms)	16.0±0.67	16.3±0.8
Rin (MΩ)	120±6.6	128±10
Spike		
Amplitudes (mV)	92.7±0.96	91.8±1.2
Half Widths (ms)	1.47±0.021	1.48±0.03
Rise Time 10-90% (ms)	0.38±0.007	0.39±0.01
Decay Time 90-10% (ms)	1.57±0.03	1.63±0.04
Threshold (mV)	-42.2±0.37	-41.8±0.46
ISI1 (ms)	10.8±0.69	11.54±1.09
ISIN (ms)	63.9±2.6	62.0±2.1
ISI1/ISIN	0.17±0.009	0.18±0.015
fAHP (mV)	-2.15±0.27	-2.16±0.25

Electrophysiological properties of pyramidal neurons in the ventromedial prefrontal cortex. No significant differences were identified in the baseline physiological characteristics of neurons infected with ROCK2 shRNA- or a scrambled control-expressing viral vector (experimental parameters are defined in (Hazra et al 2011)). See also Harbom et al., 2018. Means ± SEMs. All *p* values >0.05.

Supplementary table 2.

Figure	Ns for individual groups
1a	Saline males: 11 Saline females: 11 Fasudil males:12 Fasudil females: 12
1b	Saline (originally paired with the fasudil-treated mice): 7 Saline (originally paired with the ketamine/fluoxetine-treated mice): 19 Fasudil: 9 Ketamine: 8 Fluoxetine: 6
1c	Saline (originally paired with the fasudil-treated mice): 4 Saline (originally paired with the ketamine/fluoxetine-treated mice): 10 Fasudil: 8 Ketamine: 8 Fluoxetine: 8
1d	Vehicle: 8 SLx-2119: 9
2a	Adult: 7 fasudil mice (compared to 6 saline mice) Adolescent: 9 fasudil mice (compared to 7 saline mice)
2b	Adult: 10 fasudil mice (compared to 10 saline mice) Adolescent: 8 fasudil mice (compared to 8 saline mice)
2c	Adult: 7 fasudil (compared to 6 saline mice) Adolescent: 7 fasudil (compared to 6 saline mice)
2d	Adult: 8 fasudil (compared to 7 saline mice) Adolescent: 10 fasudil (compared to 10 saline mice)
2e	Vehicle: 7 MK-2206: 4
2f	Vehicle+saline: 7 MK-2206+saline: 6 MK-2206+fasudil: 7
2g	Adult: 8 fasudil (compared to 6 saline mice) Adolescent: 6 fasudil (compared to 6 saline mice)
2h	Adult: 8 fasudil (compared to 6 saline mice) Adolescent: 7 fasudil (compared to 5 saline mice)
3b	Adolescent saline: 7 mice (dendrites/mouse: 7,7,6,6,7,6,6) Adolescent fasudil: 7 mice (dendrites/mouse: 6,8,7,5,8,7,7) Washout saline: 6 mice (dendrites/mouse: 8,8,9,8,7,7) Washout fasudil: 6 mice (dendrites/mouse: 9,8,7,7,6,7)
3c	Adolescent saline: 7 mice (dendrites/mouse: 4,6,4,7,5,6,4) Adolescent fasudil: 7 mice (dendrites/mouse: 5,6,3,4,5,4,4)
3d	Washout saline: 6 mice (dendrites/mouse: 10,8,6,4,4,6) Washout fasudil: 6 mice (dendrites/mouse: 5,7,7,7,5,5)
3e	Adolescent saline: 6 mice (dendrites/mouse: 10,10,4,8,9,7) Adolescent fasudil: 5 mice (dendrites/mouse:10,10,9,9,10)
4b	Saline: 9 Fasudil: 9
4c	Saline: 9 Fasudil: 9
4d	Saline: 9 Fasudil: 9
4f	Saline: 9 Fasudil: 9
4g	Saline: 9 Fasudil: 9
4i	Saline: 10 Fasudil: 11

4j	Saline: 10 Fasudil: 11
4k	Saline: 10 Fasudil: 11
5b	Control: 5 shRNA: 8
5d	Control: 11 shRNA: 10
5e	Control: 49 cells (from 9 mice) shRNA: 52 cells (from 10 mice)

Group sizes for individual experimental groups reported in the main text. Note that when an experiment contained multiple saline control groups, these groups did not differ and are combined in the main document.

Supplementary table 3.

Figure	Numbers of mice excluded per experimental group (exclusions due to outlying values unless otherwise noted)
1a	Saline males: 0 Saline females: 0 Fasudil males: 1 Fasudil females: 1
1b	Saline (originally paired with the fasudil-treated mice): 1 Saline (originally paired with the ketamine/fluoxetine-treated mice): 1 Fasudil: 0 Ketamine: 0 Fluoxetine: 0
1c	Saline (originally paired with the fasudil-treated mice): 0 Saline (originally paired with the ketamine/fluoxetine-treated mice): 1 Fasudil: 0 Ketamine: 1 Fluoxetine: 0
1d	Vehicle: 2 SLx-2119: 1
2a	Adult: 0 Adolescent: 0 (1 saline mouse removed prior to normalization)
2b	Adult: 0 Adolescent: 0
2c	Adult: 0 Adolescent: 0
2d	Adult: 0 Adolescent: 0
2e	Adult: 0 Adolescent: 0
2f	Vehicle+saline: 0 MK-2206+saline: 0 MK-2206+fasudil: 0
2g	Adult: 0 Adolescent: 1
2h	Adult: 0 Adolescent: 0 (1 saline mouse removed prior to normalization)
3b	Adolescent saline: 0 Adolescent fasudil: 0 Washout saline: 0 Washout fasudil: 0
3c	Adolescent saline: 0 Adolescent fasudil: 0
3d	Washout saline: 0 Washout fasudil: 0
3e	Adolescent saline: 0 Adolescent fasudil: 0
4b	Saline: 0 Fasudil: 0
4c	Saline: 0 Fasudil: 0
4d	Saline: 0 Fasudil: 0
4f	Saline: 0 Fasudil: 0
4g	Saline: 0 Fasudil: 0
4i	Saline: 1 Fasudil: 0

4j	Saline: 1 Fasudil: 0
4k	Saline: 1 Fasudil: 0
5b	Control: 0 shRNA:0
5d	Control: 7 shRNA: 7 These mice were excluded due to unilateral viral vector infection, ventral/caudal infection that extended into the striatum, or rostral infection that only encompassed the medial orbitofrontal cortex
5e	Control: 5 cells, 0 mice shRNA: 3 cells, 0 mice

Numbers of mice excluded per experimental group reported in the main text. Note that when an experiment contained multiple saline control groups, these groups did not differ and are combined in the main document.

Supplementary Works Cited

Harbom LJ, Rudisill TL, Michel N, Litwa KA, Beenhakker MP, McConnell MJ. 2018. The effect of rho kinase inhbtion on morphological and electrophysiological maturity in iPSC-derived neurons. *Cell Tissue Res*, epub ahead of print

Hazra R, Guo JD, Ryan SJ, Jasnow AM, Dabrowska J, Rainnie DG. 2011. A transcriptomic analysis of type I-III neurons in the bed nucleus of the stria terminalis. *Mol Cell Neurosci* 46: 699-709

Pitts EG, LI DC, Gourley SL. 2018. Bidirectional coordination of actions and habits by TrkB in mice. *Sci Rep* 8: 4495

Rattiner LM, Davis M, French CT, Ressler KJ. 2004. Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 24: 4796-806