Sestrin2 modulator NV-5138, shows ketamine-like rapid antidepressant effects via direct activation of mTORC1 signaling

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Introduction

In preclinical rodent models the antidepressant actions of ketamine are dependent on mTORC1 signaling. The mTORC1 pathway is modulated by neuronal activity, endocrine and metabolic signals, including amino acids, notably leucine, which activates mTORC1 signaling via binding to sestrin2 (Wolfson and Sabatini, 2017). Here, we examined the influence of NV-5138, a small molecule modulator of sestrin2 that penetrates the blood brain barrier and causes activation of mTORC1 on antidepressant behavioral responses and induction of spine synapses in the prefrontal cortex (PFC).

The results demonstrate that a single dose of NV-5138 produced rapid antidepressant effects in rodent models and rapidly reversed the anhedonia caused by chronic unpredictable stress. The effects of NV-5138 were long-lasting (up to 7 d) following a single dose. As expected, NV-5138 activated mTORC1 and the antidepressant actions of NV-5138 were blocked by infusion of the selective mTORC1 inhibitor rapamycin into mPFC. We also found that NV-5138 rapidly increased the number and function of spine synapses of mPFC apical dendrites.

Taken together, the results demonstrate that NV-5138 produces rapid synaptic and antidepressant behavioral responses via direct activation of mTORC1 signaling, supporting the possibility that sestrin2 modulation is a novel target for development of rapid acting antidepressants.

Materials & Methods

Animals: Male Sprague-Dawley rats were used. NV-5138 was dissolved in 0.5%methyl cellulose with 0.1% tween80 and administered orally. Ketamine was dissolved in saline and injected intraperitoneally. Animal use and procedures were in accordance with the National Institutes of Health guidelines and approved by the Yale University Animal Care and Use Committees.

Surgery and intra-mPFC infusion: Rats were anaesthetized with 80 mg ketamine/6 mg/kg xylazine i.p., and bilateral 22-guage guide cannula were implanted at 0.5 mm above the site of infusion (+3.0 mm AP; ± 1.0 mm ML; -4.0 mm DV to the bregma). Following 13 or 14 day of recovery, rats were bilaterally infused with a rapamycin (0.005 nmol/side) or 10% DMSO.

Behavioral testing:

Forced swim test; Rats but not mice were experienced preswim 24 hr before drug treatment. Twenty-four hour after drug treatment, rats or mice were underwent a 10-min test swim. Sessions were video recorded and data analyzed by blind manner.

Female urine sniffing test; Animals were habituated for 60 min to a cotton-tipped applicator dipped in tap water placed in their home cage. For the test, animals were first exposed to a new cotton tip dipped in tap water for 5 min. Forty five minute later, animals were exposed to another cotton tip infused with fresh urine collected from females for 5 min. Male behavior was video recorded and total time spent sniffing the cotton-tipped applicator were determined.

Novelty suppressed feeding test; Animals were food-deprived overnight and placed in an open field with a small amount of food in the center. The latency to feed was measured with a cut-off time of 15 min. After the NSF, home cage feeding (HCF) during a 10-min period was measured to verify motivation to feed.

Locomotor activity; Number of beam break were measured for 30 min in an empty rat cage equipped with automated activity meters (Digiscan animal activity monitor; Omnitech Electronics, Columbus, Ohio) as previously described (Warner-Schmidt and Duman, 2007).

Sucrose preference test; Rats were exposed to a palatable sucrose solution (1%; Sigma) for 48 hrs, followed by 4 hrs of water deprivation and a 1 hr exposure to two identical bottles, one filled with sucrose solution and the other with water. Sucrose and water consumption were determined by measuring the change in the volume of fluid consumed. Sucrose preference was defined as the ratio of the volume of sucrose versus total volume of sucrose and water consumed during the 1 hr test.

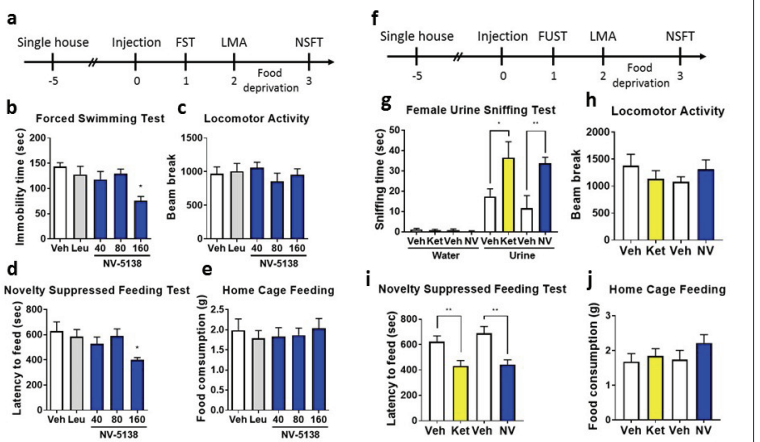
CUS procedure: Animals were exposed to a variable sequence of mild and unpredictable stressors for 26 days, a procedure that we have found produces depressive-like behavioral changes. A total of 11 different stressors were used (2 stressors per day). The stressors included forced swim, rotation on a shaker, placement in a 4 ºC ambient, lights off for 3 hours (10:00 AM–1:00 PM) or on overnight, strobe light overnight, white noise over night, wet or no bedding, 45˚ tilted cages, food and water deprivation, crowded housing, and restraint in plastic bag.

Western Blot: To purify a crude synaptoneurosome, prefrontal cortex, hippocampus or striatum from adult rats were dissected and homogenized in a solution containing 0.32 M sucrose, 20 mM HEPES (pH 7.4), 1 mM EDTA, 1X protease inhibitor cocktail, 5 mM NaF, and 1 mM sodium vanadate. The homogenate was centrifuged for 10 min at 2,800 rpm at 4 ºC. The pellet (nuclear fraction) contains nuclei and large cell debris. The supernatant was centrifuged at 12,000 rpm for 10 min. After centrifugation, the supernatant (cytosolic fraction) was removed and the pellet (crude synaptosomal fraction) was resuspended and sonicated in protein lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 2 mM EDTA, 1 mM $NaVO_3$, 5 mM NaF and 1X protease inhibitor cocktail). For western blotting, equal amounts of protein (20 µg) for each sample were loaded into 4-20% SDS PAGE gel for electrophoresis. Polyvinylidene difluoride (PVDF) membranes with transferred proteins were blocked with 5% BSA or 5% skim milk in PBST (PBS + 0.1% Tween-20) for 1 hr and kept with primary antibodies overnight at 4 ºC. The next day, blots were incubated with horseradish peroxidase conjugated anti-rabbit secondary antibody (1:5000 to 1:10000) for 1 hr. Bands were detected using enhanced chemiluminescence (ECL).

Electrophysiological Recordings and spine analysis: One day after drug treatment, rats were anesthetized and brains were removed. Coronal slices 400-µm thick were cut in ice-cold sucrose–ACSF from a block of tissue containing the mPFC with an oscillating-blade tissue slicer. Slices were placed in a submerged recording chamber; bath temperature was then raised slowly to 32 ºC. The standard ACSF (pH 7.35), equilibrated with 95% O_2/5% CO_2, contained 128 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgSO_4$, 24 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, and 10 mM, D-glucose. There was recovery period of 1-2 hr before recording. Pyramidal neurons in layer V were patched under visual control using infrared differential interference contrast (IR/DIC) microscopy. Patch pipettes (3-5 MΩ) were pulled from glass tubing with a Flaming-Brown Horizontal Puller. 115 mM K gluconate, 5 mM KCl, 2 mM $MgCl_2$, 2 mM Mg-ATP, 2 mM Na_2ATP, 10 mM Na_2-phosphocreatine, 0.4 mM Na_2GTP, and 10 mM HEPES, pH 7.33. Neurobiotin (0.3%) was added to the pipette solution to mark cells for later processing and imaging. The output signal was low-pass-filtered at 3 KHz and digitized at 15 kHz; data were acquired by pClamp 10.5/Digidata 1550 A software. After completion of recording, slices were transferred to 4% paraformaldehyde and stored overnight at 4 ºC. Slices were then processed with Streptavidin conjugated to Alexa 594 (1:500) for visualization of labeled cells.

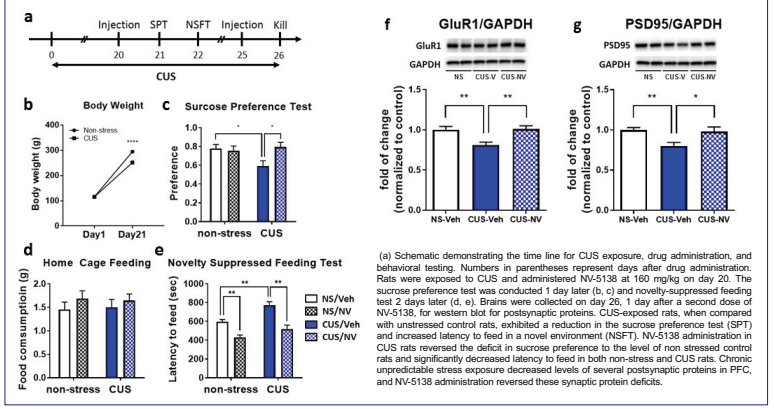
Results

Fig. 1 NV-5138 shows ketamine-like antidepressant actions in rodent models of depression



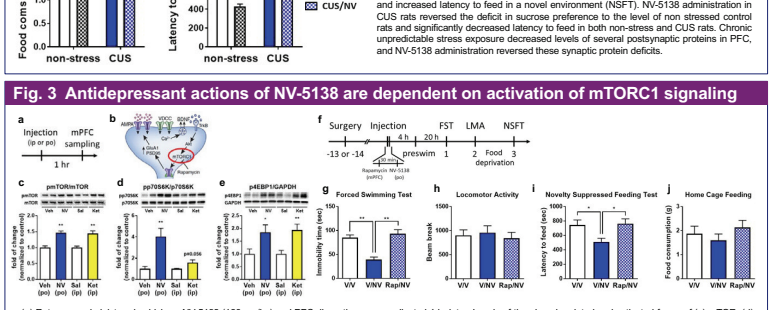
(a) Twenty-four hour after the administration of NV-5138 (40, 80, or 160 mg/kg p.o.) or leucine at 160 mg/kg behavioral studies were conducted over the next 3 days (b-e). Significant effects of NV-5138 were observed at 160 mg/kg in (b) the FST and (d) the NSFT. No significant effects were seen in (c) locomotor activity or (e) home cage feeding. (f) Twenty-four hour after ketamine (10 mg/kg i.p.) or NV-5138 (160 mg/kg) administration behavioral studies were conducted over the next 3 days (g-j). Significant effects of ketamine and NV-5138 were observed in (g) the FUST and (i) the NSFT. No significant effects were seen in (h) locomotor activity or (j) home cage feeding.

Fig. 2 NV-5138 Reverses the Behavioral and Synaptic Deficits Caused by CUS



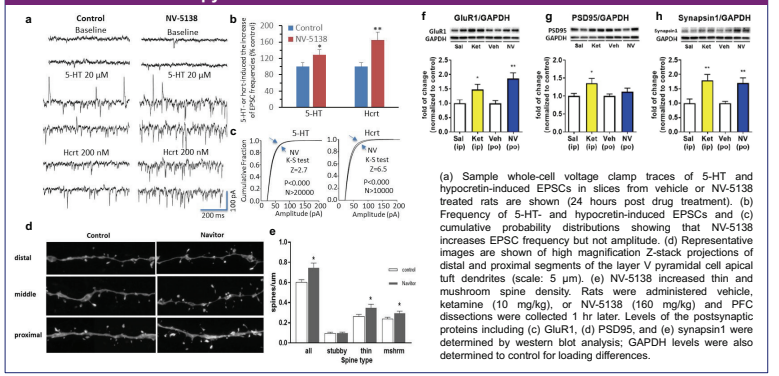
(a) Schematic demonstrating the time line for CUS exposure, drug administration, and behavioral testing. Numbers in parentheses represent days after drug administration. Rats were exposed to CUS and administered NV-5138 at 160 mg/kg on day 20. The sucrose preference test was conducted 1 day later (b, c) and novelty-suppressed feeding test 2 days later (d, e). Brains were collected on day 26, 1 day after a second dose of NV-5138, for western blot for postsynaptic proteins. CUS-exposed rats, when compared with unstressed control rats, exhibited a reduction in the sucrose preference test (SPT) and increased latency to feed in a novel environment (NSFT). NV-5138 administration in CUS rats reversed the deficit in sucrose preference to the level of non stressed control rats and significantly decreased latency to feed in both non-stress and CUS rats. Chronic unpredictable stress exposure decreased levels of several postsynaptic proteins in PFC, and NV-5138 administration reversed these synaptic protein deficits.

Fig. 3 Antidepressant actions of NV-5138 are dependent on activation of mTORC1 signaling



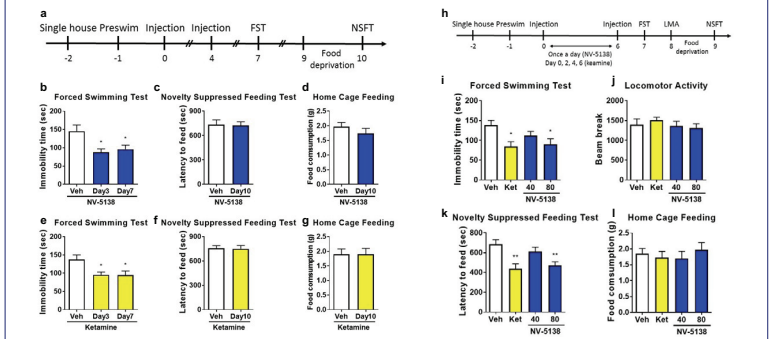
(a) Rats were administered vehicle or NV-5138 (160 mg/kg) and PFC dissections were collected 1 hr later. Levels of the phosphorylated and activated forms of (c) mTOR, (d) p70S6K, and (e) 4EBP1 were determined by western blot analysis; levels of GAPDH were also determined to control for loading differences. (f) Rats were implanted with bilateral cannula in the mPFC and allowed to recover for approximately 2 weeks. The mTORC1 inhibitor rapamycin was infused into the mPFC 30 min prior to administration of vehicle or NV-5138. Twenty-four hr after NV-5138 administration behavioral studies were initiated and conducted over the next 3 days (g-j). Significant effects of NV-5138 were blocked by rapamycin in c) the FST and (e) the NSFT. No significant effects were seen in (h) locomotor activity or (j) home cage feeding.

Fig. 4 NV-5138 increases synaptic proteins, spine number, and hypocretin- and 5-HT-induced EPSCs in mPFC pyramidal neurons



(a) Sample whole-cell voltage clamp traces of 5-HT and hypocretin-induced EPSCs in slices from vehicle or NV-5138 treated rats are shown (24 hours post drug treatment). (b) Frequency of 5-HT- and hypocretin-induced EPSCs and (c) cumulative probability distributions showing that NV-5138 increases EPSC frequency but not amplitude. (d) Representative images are shown of high magnification Z-stack projections of distal and proximal segments of the layer V pyramidal cell apical tuft dendrites (scale: 5 µm). (e) NV-5138 increased thin and mushroom spine density. Rats were administered vehicle, ketamine (10 mg/kg), or NV-5138 (160 mg/kg) and PFC dissections were collected 1 hr later. Levels of the postsynaptic proteins including (c) GluR1, (d) PSD95, and (e) synapsin1 were determined by western blot analysis; GAPDH levels were also determined to control for loading differences.

Fig. 5 Single dose NV-5183 (160 mg/kg) produces long-lasting antidepressant effects, similar to ketamine; repeated low dose NV-5138 (80 mg/kg) also produces antidepressant effects



(a) Twenty-four hour after NV-5138 (160 mg/kg) or ketamine (10 mg/kg) administration behavioral studies were conducted on day 3, 7 and 10 later to evaluate the duration of action (b-g). Significant effects of NV-5138 and ketamine were observed in (b,e) the FST on day **3** and 7 but not in (c,f) the NSFT on day 10. No significant effects were seen in (d,g) home cage feeding by either drug treatment. (h) Low dose NV-5138 was administered once a day for 7 days and ketamine was administered on alternate days over the same time frame. Twenty-four hour after last administration, behavioral studies were conducted over the next 3 days (i-l). Significant effects of NV-5138 were observed at 80 mg/kg in (i) the FST and (k) the NSFT. No significant effects were seen in (j) locomotor activity or (l) home cage feeding. Ketamine produced significant effects in both behavior tests.

Summary

- Orally administered NV-5138 produces rapid, dose dependent antidepressant effects in several rodent models.
- NV-5138 reverses the synaptic and behavioral deficits caused by exposure to CUS, similar to ketamine.
- NV-5138 enhances the phosphorylation and activation of mTORC1 related signaling proteins as expected, and intra-mPFC infusion of the mTORC1 selective inhibitor rapamycin blocked the antidepressant effects of NV-5138.
- NV-5138 increases spine density and enhances synaptic function of mPFC layer V pyramidal neurons.
- The antidepressant effects of a single dose of NV-5138 in rat models are sustained up to 7 days, similar to ketamine.

The results demonstrate that NV-5138 produces rapid synaptic and antidepressant behavioral responses via direct activation of the mTORC1 signaling pathway, supporting the possibility that sestrin2 modulation is a novel target for development of rapid acting antidepressants. Current studies are being conducted to determine whether the mechanisms underlying the action of NV-5138 include BDNF-TrkB, similar to the effects of ketamine, as well as other rapid acting agents, including GLYX-13 and scopolamine. These studies will further characterize the cellular mechanisms underlying the actions of NV-5138 and highlight common molecular mechanisms of rapid acting agents.

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