**Knocking Out Addiction: Characterizing NPS Antagonism**

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**Introduction**

Emerging data suggest that neuropeptide S (NPS), a recently discovered peptide, may be important in behaviors relevant to drug addiction [1]. NPS receptor (NPSR) mRNA is expressed in brain regions implicated in drug seeking and reinstatement, while NPS mRNA is predominantly expressed in glutamatergic cells.

Recent findings in the literature have shown evidence that NPS increases cue-induced cocaine and ethanol seeking, stress-induced ethanol seeking, and that NPS reinstates cocaine and ethanol seeking by itself [2, 3].

Based on the fact that NPS increases the release of glutamate and increases in glutamate are implicated in drug seeking, we hypothesized that an NPSR antagonist (SHA-68) should decrease glutamate release in the nucleus accumbens (NAc) and therefore blunt drug seeking behavior.

Glutamate was measured in the NAc and prefrontal cortex (PFC) using biosensor technology. We took advantage of the NPSR KO mice to demonstrate that the effect of SHA-68 on glutamate release is mediated by NPSR.

In addition, we studied the effect of the NMDA receptor antagonist MK-801 on glutamate release in the NAc and PFC of WT and NPSR KO mice.

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**Methods**

**Animals / Drug**

- Male C57BL/6 and NPSR KO mice (>80 days) from Jackson Laboratory were used.
- SHA-68 Selective brain penetrant NPSR antagonist Kᵢ=11 nM

**Biosensor – Glutamate**

- Each mouse was implanted with a cannulae (BAS) in the NAc or PFC (NAc: AP = 1.34, ML = 1.0, V = 3.8 mm PFC: AP = 1.54, ML = 0.5, V = 2.0 mm, respectively)
- The day prior to experimentation animals were implanted with a biosensor (1 mm active membrane length) into the NAc or PFC (Pinnacle Technologies).
- The following morning each animal received a vehicle injection i.p. then 90-min later injected with SHA-68 at 50 mg/kg i.p., subsequently each animal received a s.c. injection of MK-801 at 0.178 mg/kg as a positive control. For MK-801 alone studies, each animal received a s.c. vehicle injection followed by a s.c. injection of MK-801 at 0.178 mg/kg.
- Upon implantation, each sensor was calibrated in a circulating water bath at 37°C for glutamate response and ascorbate rejection.

**Amphetamine-Induced Condition Place Preference (CPP)**

- WT or NPSR KO mice were conditioned to amphetamine (2 mg/kg) for 4 days in a two-chamber, unbiased conditioned place preference (CPP) chamber.
- In a separate experiment, KO mice were conditioned to naltrexone (10 mg/kg) using similar methods.
- A separate group of WT mice were conditioned to amphetamine and given SHA-68 (50 mg/kg, i.p.) or vehicle 15 min before the CPP test.

**Results**

- The NPSR antagonist decreases glutamate release in the NAc of WT mice, but not NPSR KO mice.
- The NMDA receptor antagonist increases glutamate release in the NAc of WT mice, but not NPSR KO mice.
- The NPSR antagonist does not change glutamate release in the PFC of WT mice.
- The NMDA receptor antagonist increases glutamate release in the PFC of WT and NPSR KO mice.

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**Conclusions**

- Blockade of NPSR decreased glutamate in the NAc. Interestingly, this effect was selective to the NAc as NPSR antagonism had no effect on cortical glutamate release. Similarly, the NMDA receptor antagonist increased GLU in the cortex, but not the NAc, of NPSR KO mice.
- In a separate study, we showed that NPS increased dopamine release in rat PFC [4]. Therefore, the effect of the NPSR antagonist on glutamate could be driven by cortical afferents.
- NPS selectively modulates glutamate in the NAc, an area vital to drug-seeking behaviors, and is required for the glutamate-releasing effects of psychostimulants. This blunted glutamate signaling in the NAc after NPSR blockade or knockout may be responsible for the observed CPP results.
- Cumulatively, these findings suggest that NPSR antagonism may provide a novel treatment for drug seeking and abuse.

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**References**


2. Sato K et al. (2008) Molecular Pharmacology 73, 238-241

3. Sato K et al. (2008) Molecular Pharmacology 73, 238-241