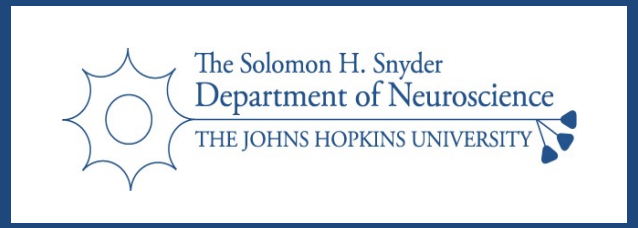

Cocaine Receptor Identified as BASP1

Undergraduate Research Symposium

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Introduction

- Cocaine is known to be a behavioral stimulant with significant abuse potential.
- Inhibit the reuptake activation of neurotransmitters such as dopamine, serotonin, and norepinephrine at high nanomolar to low micromolar concentrations.
- There have been reports on more potent influences of cocaine that require high affinity actions:
  - Environmental place conditioning in planarians (Tallarida et al., 2014).
  - Cocaine binding to brain membranes with a dissociation constant of ~16nM (Calligaro & Eldefrawi, 1987).
- Here, we report high affinity binding of cocaine to the membrane-associated brain acid soluble protein-1 (BASP1).

Materials & Methods

- Reagents:
  - Anisodamine, benzocaine, benzoylecgonine, benztropine, 3-p-FBT (hydrochloride), procaine, and tropine
  - Cocaine hydrochloride, atropine, benzoic acid, benztropine, ecgonine, lidocaine, scopolamine and glutathione agarose
- Ligand binding assay
  - Synaptosome fraction
- Cocaine immunoprecipitation
  - LCMS/MS analysis
  - Transient Polyfect and lipofectamine 3000 transfection
- Primary Cortical Neuron culture
- Locomotor sensitization experiments
  - Open field test
  - Photobeam activity system, SDI PAS-Open Field software, San Diego Instruments

Results

Identification of cocaine binding proteins

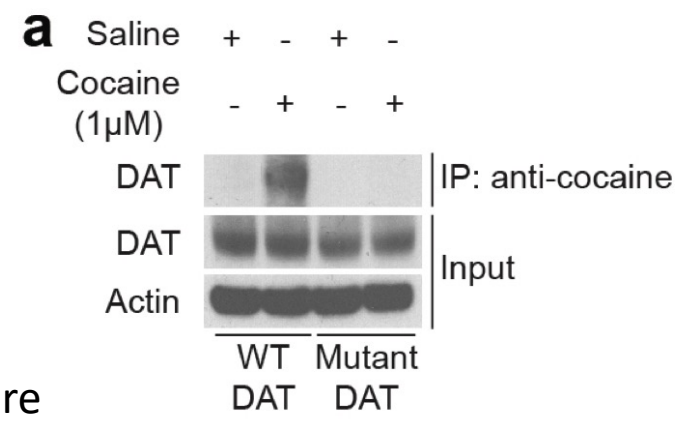
Two groups of HEK 293 cells:

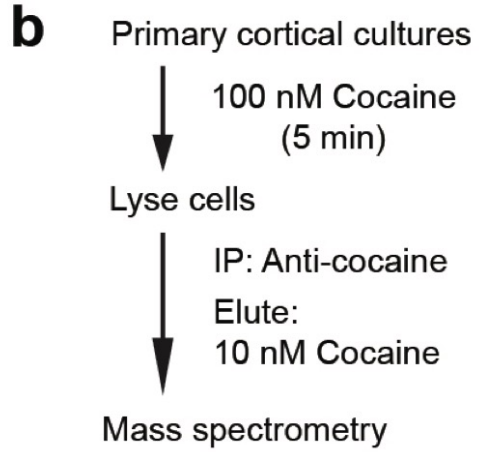
- WT DAT
- L104V-F105C-A109V triple mutant DAT
  (insensitive to cocaine)

Input of DAT and actin serve as loading controls

Mass spectrometry analysis reveals six proteins were pulled down

- Exclusion of common contaminants
- BASP1 as a **putative receptor for cocaine**, because it is the only membrane-associated protein

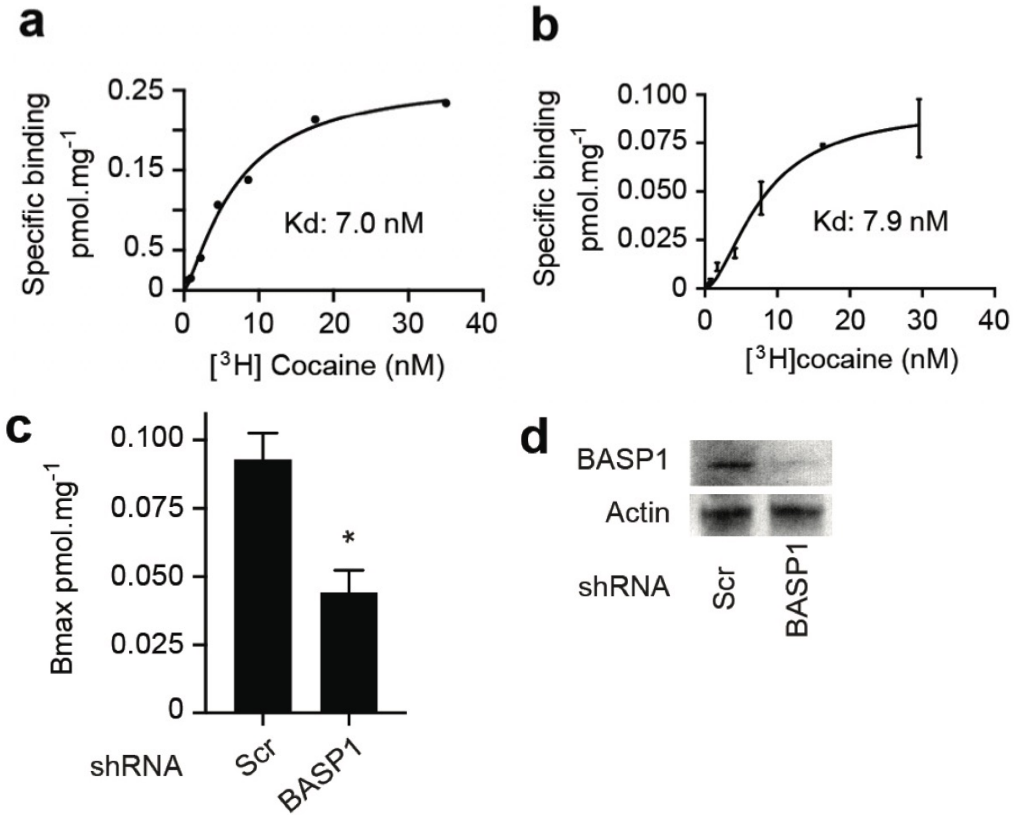




Results

BASP1 is a high affinity cocaine binding protein

- Overexpressed BASP1
  - $Kd = 7.0\ nM$
- Rat striatal synaptosomal fraction
  - $Kd = 7.9\ nM$
- BASP1 depletion by shRNA
  - ~50% decrease in BASP1
  - ~50% decrease in Bmax

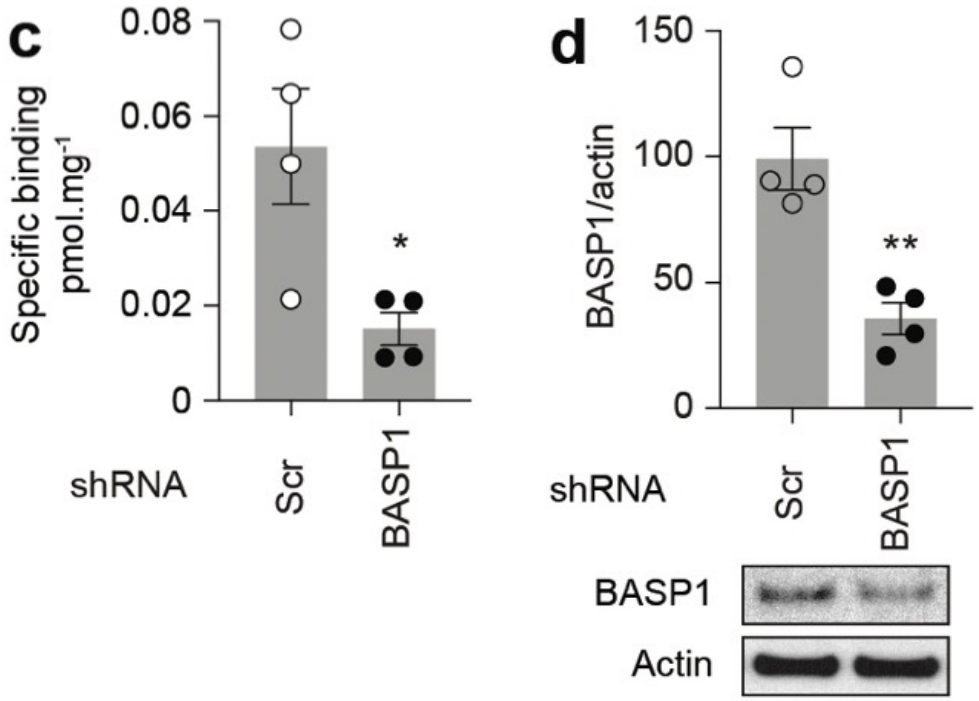


Results

BASP1 is a high affinity cocaine binding protein

In mouse striatal synaptosomal fraction

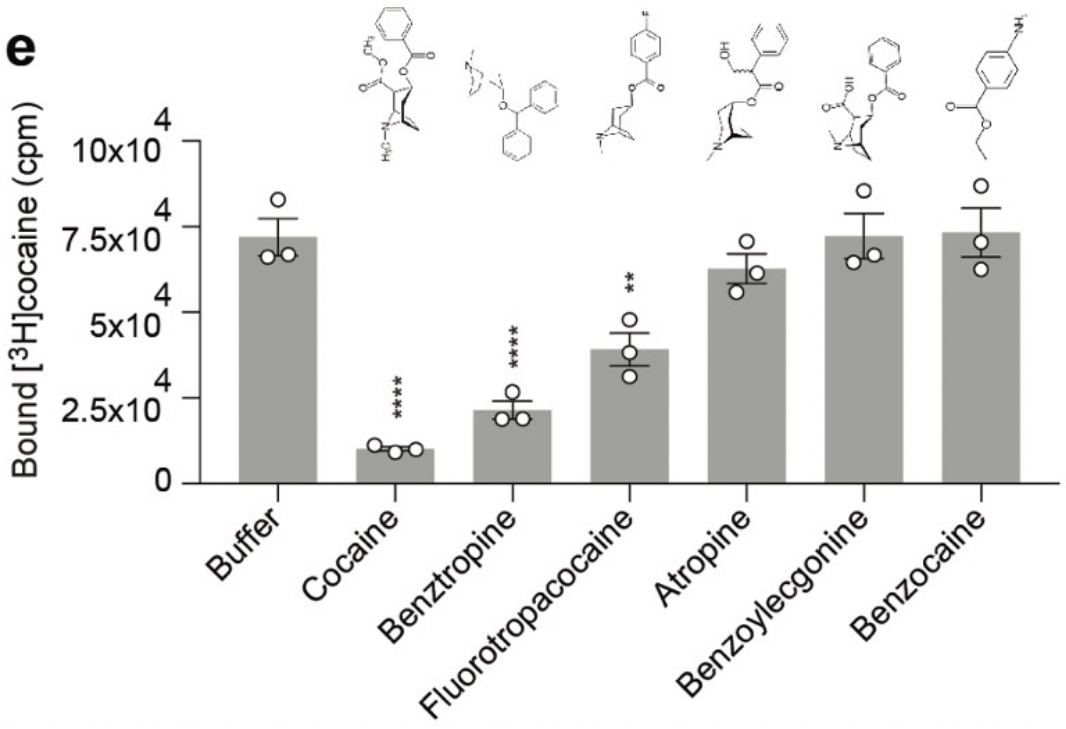
- Depletion of BASP1 by shRNA treatment
  - Decreased specific binding
  - Diminished Bmax
  - Unaffected Kd



Results

BASP1 is a high affinity cocaine binding protein

- Only Benzotropine and 3-p-FBT elute [^{3}H] cocaine from BASP1 containing membranes.
- Major cocaine metabolite benzoylecgonine does not dissociate [^{3}H] cocaine from BASP1.

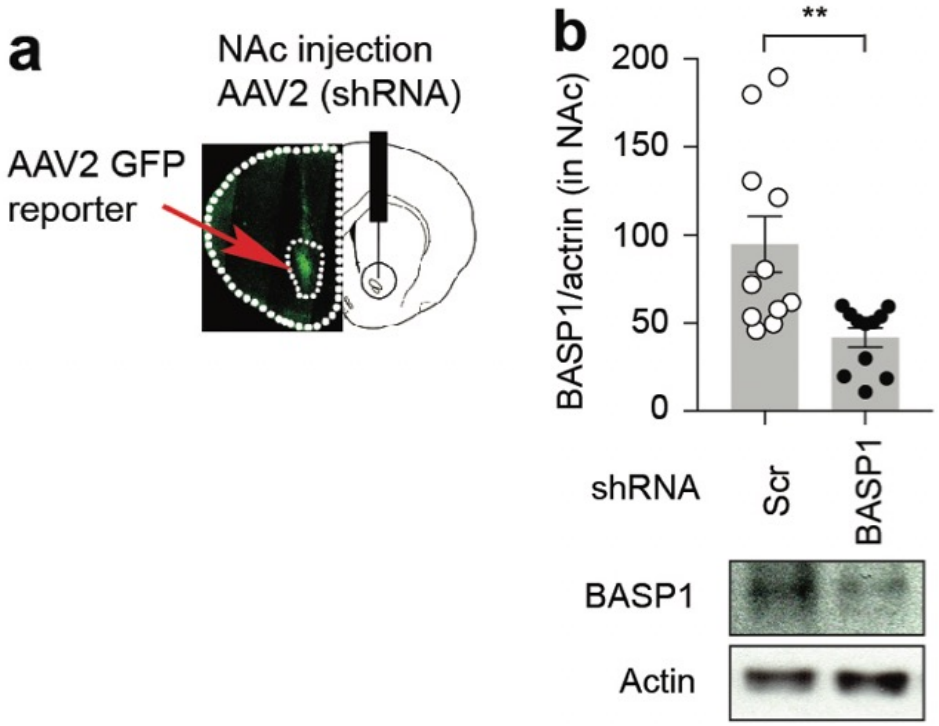


Results

BASP1 mediates the locomotor stimulant effect of cocaine.

Outline of the experiment:

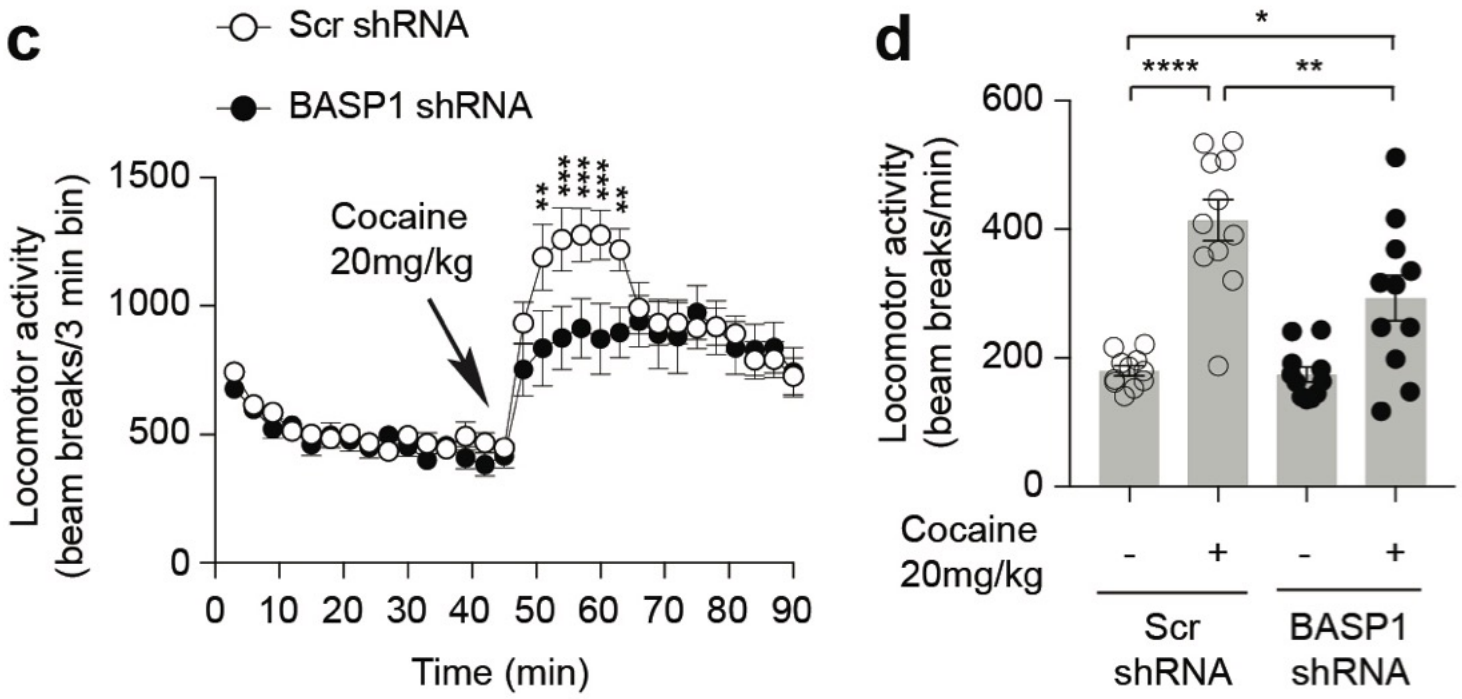
1. shRNA treatment two weeks prior to the experiment (figure a,b)
   - Scr shRNA
   - BASP1 shRNA
2. Baseline activity was measured for the first 45 minutes
3. After the i.p injection of 20mg/kg of cocaine (saline for control), their locomotor behavior was measured.



Results

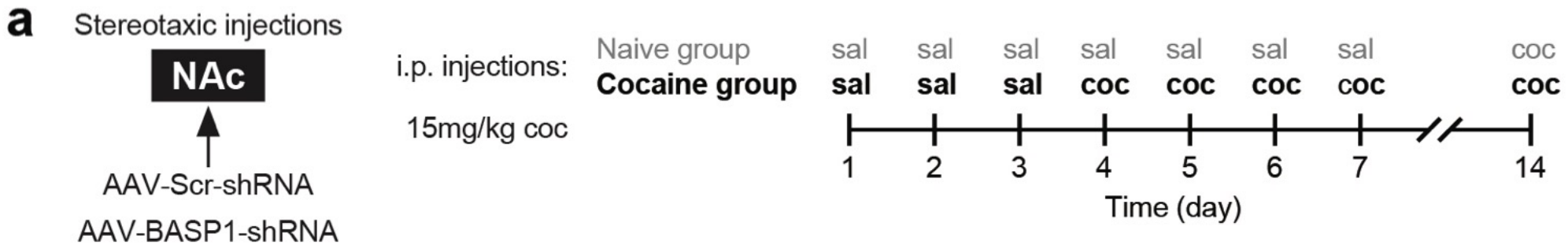
BASP1 mediates the locomotor stimulant effect of cocaine

- BASP1 depletion without cocaine treatment does not affect spontaneous locomotor activity in mice.
- BASP1 depletion decreases the stimulatory actions of cocaine compared to scrambled RNA.



Results

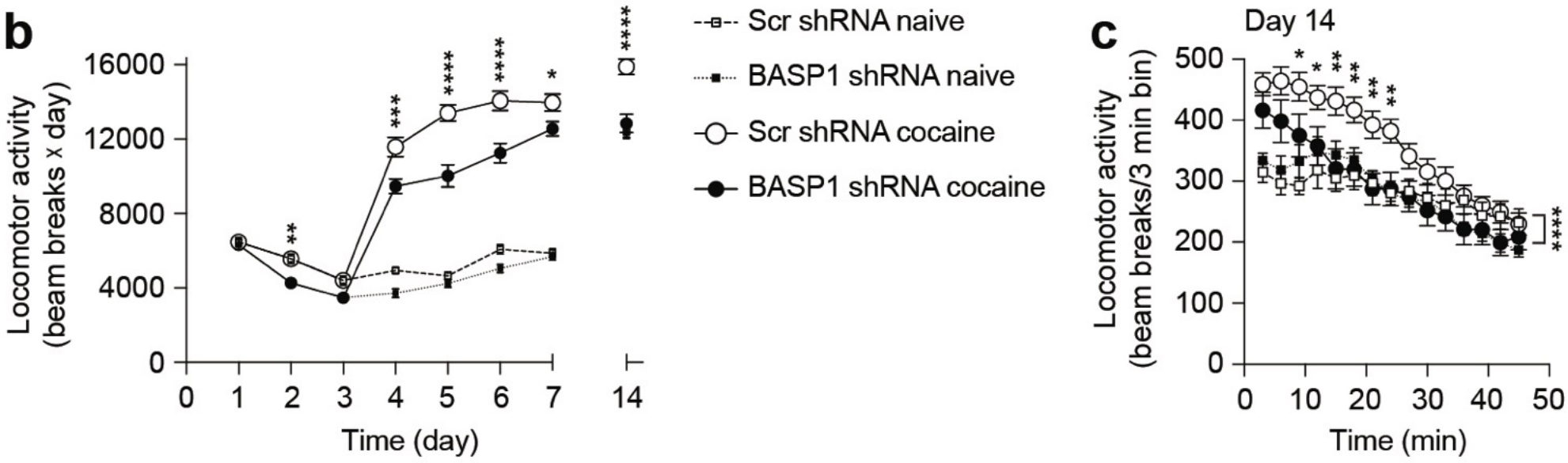
BASP1 regulates the acquisition and expression of locomotor sensitization to cocaine



Locomotor sensitization: Progressive and long-lasting increase in locomotor stimulant effect triggered by a later drug injection due to repeated cocaine administration.

Results

BASP1 regulates the acquisition and expression of locomotor sensitization to cocaine



Summary

- **Identification of cocaine binding proteins.**
- **BASP1 is a high affinity cocaine binding protein.**
- **BASP1 mediates the locomotor stimulant effect of cocaine.**
- **BASP1 regulates the acquisition and expression of locomotor sensitization to cocaine**

References

- Calligaro, D. O. & Eldefrawi, M. E. Central and peripheral cocaine receptors. *J. Pharmacol. Exp. Ther.* **243,** 61–68 (1987).
- Tallarida, C. S. *et al.* Ethanol and cocaine: environmental place conditioning, stereotypy, and synergism in planarians. *Alcohol* **48,** 579–586 (2014).