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Short communication

Developmentally divergent effects of Rho-kinase inhibition on cocaine- and BDNF-induced behavioral plasticity

Lauren M. DePoy[a,b,c], Benjamin Noble[a,b], Amanda G. Allen[a,b], Shannon L. Gourley[a,b,c,*]

[a] Department of Pediatrics, Emory School of Medicine, Atlanta, GA, United States
[b] Yerkes National Primate Research Center, Atlanta, GA, United States
[c] Graduate Program in Neuroscience, Emory University, Atlanta, GA, United States

HIGHLIGHTS

- Orbitofrontal cortical dendritic spines proliferate and refine in adolescence.
- Structural instability in adolescence exaggerates adult cocaine sensitivity.
- By contrast, destabilizing spines in adulthood can have behavioral benefits.
- For example, Rho-kinase inhibition blocks 'reward seeking' after orbital *Bdnf* knockdown.

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ABSTRACT

Prefrontal cortical dendritic spine remodeling during adolescence may open a window of vulnerability to pathological stimuli that impact long-term behavioral outcomes, but causal mechanisms remain unclear. We administered the Rho-kinase inhibitor HA-1077 during three adolescent periods in mice to destabilize dendritic spines. In adulthood, cocaine-induced locomotor activity was exaggerated. By contrast, when administered in adulthood, HA-1077 had no psychomotor consequences and *normalized* food-reinforced instrumental responding after orbitofrontal-selective knockdown of *Brain-derived neurotrophic factor*, a potential factor in addiction. Thus, early-life Rho-kinase inhibition confers cocaine vulnerability, but may actually protect against pathological reward-seeking – particularly in cases of diminished neurotrophic support – in adulthood.

1. Prefrontal cortical dendritic spines remodel in adolescence

Adolescence marks a neurobiologically distinct developmental period characterized by high rates of experimental drug use and vulnerability to the development of substance dependence. Adolescent substance exposure markedly increases the likelihood of developing lifelong dependence, defined by a loss of control over drug intake and drug use despite adverse consequences [1,2]. Cocaine dependence emerges with particular virulence; for example, approximately 15–16% of adolescent cocaine users will develop dependence within 10 years of first exposure, but only 12–13% of alcohol users and 8% of marijuana users will develop dependence in the same period [3]. Thus, identifying mechanisms of cocaine vulnerability is a critical research imperative.

It is widely appreciated that amphetamine and amphetamine-like psychostimulants such as cocaine are potent regulators of dendritic spines within the prefrontal cortex [4]. In the orbitofrontal prefrontal cortex (oPFC), a structure widely associated with addiction vulnerability [5], exposure to psychostimulants decreases dendritic spine density [6–8]. Moreover, cocaine exposure in adolescence causes oPFC dendritic spine elimination that is sustained into adulthood [8], suggesting early-life structural modifications may contribute to lifelong vulnerability to cocaine.

Recent work with genetic knockout models supports this perspective: Mice deficient in beta1-integrin, p190RhoGAP, and Arg kinase, all of which are expressed in the prefrontal cortex and

Abbreviations: oPFC, Orbitofrontal prefrontal cortex; BDNF, Brain-derived Neurotrophic Factor; P, Postnatal day; GFP, Green Fluorescent Protein; NA, Numerical aperture; ROCK, Rho-kinase; ANOVA, Analysis of variance; AP, Anterior–posterior; ML, Medial-lateral; DV, Dorso-ventral; PBS, Phosphate-buffered saline.

* Corresponding author at: Yerkes National Primate Research Center, Emory University, 954 Gatewood Dr. NE, Atlanta GA 30329, United States.
Tel.: +1 404 727 2482.
E-mail address: shannon.l.gourley@emory.edu (S.L. Gourley).

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organize or are implicated in postnatal dendritic spine stability, show exaggerated cocaine-induced psychomotor sensitization in adulthood [8–11]. In the case of Arg, knockout also confers vulnerability to cocaine-induced oPFC-dependent cognitive deficits and increases sensitivity to reward-predictive cues [8,9]. A limitation of these models, however, is that unlike with viral vector approaches (*e.g.*, [12]), anatomical specificity is limited. Evidence that the spine-associated neurotrophin Brain-derived Neurotrophic Factor (BDNF) has differential effects on reward sensitivity depending on regional expression patterns reinforces the limitation of whole-brain knockout approaches (reviewed [10]). Moreover, the brief but distinct phases of adolescent prefrontal cortical dendritic spine refinement cannot be selectively manipulated using traditional knockout approaches, thus the relative contributions of dendritic spine proliferation (in early adolescence) and elimination (in mid- and late adolescence) to long-term behavioral outcomes remain unclear.

To illustrate these distinct phases here, we enumerated dendritic spines in mice expressing *thy1*-derived Green Fluorescent Protein (GFP) in deep-layer oPFC [13]. Mounted, 40-μm-thick sections were imaged using a Fluoview confocal microscope, 100× 1.4NA objective, laser excitation at 488 nm, and 0.5 μm step sizes. Collapsed z-stacks collected from the ventral/lateral compartments of the oPFC (50–150 μm from the soma) were enumerated using ImageJ. 18 independent neurons from mice aged 24, 31, and 56 days were scored, corresponding to pre-adolescence, early adolescence, and early adulthood [14]. Spine density was highest at postnatal day (P) 31 [$F(2,51) = 3.7$, $p = 0.03$], and curve fits highlight dendritic spine proliferation between P24–31 and elimination after P31 (Fig. 1a). These spine counts replicate our previous findings [8] and parallel gross volumetric changes in the rodent oPFC showing that the oPFC is largest at P31 [15].

What is the long-term significance of distinct phases of adolescent dendritic spine reorganization?

Next, we inhibited Rho-associated protein kinase II (also called Rho-kinase, or "ROCK"), a substrate of the master cytoskeletal regulator RhoA (Rho). Rho functions as a molecular switch, cycling between an inactive GDP-bound state and an active GTP-bound state in which Rho is targeted to cellular membranes. There, Rho orchestrates the formation of stress fibers and focal adhesions necessary to reorganize cellular membranes through ROCK. Thus, ROCK inhibitors interfere with activity-dependent dendritic spine remodeling (*e.g.*, [16]).

Here, male C57BL/6 mice bred in-house from Jackson Labs stock were injected with the brain-penetrant ROCK inhibitor HA-1077 (30 mg/kg, *i.p.*, LC Labs) or PBS vehicle from P24–28 or P31–35, corresponding to periods of spine proliferation and elimination during early adolescence. We also injected mice from P42–46, corresponding to the onset of the peri-adolescent period [14]. Mice were left undisturbed until adulthood (P56) when cocaine-induced psychomotor sensitization was tested as an assay of cocaine-induced behavioral plasticity.

We used a within-subjects experimental design described previously [8,9]: Mice were habituated for 1 h to Med-Associates locomotor monitoring chambers (41 × 20 × 20 cm), then injected with cocaine HCl (10 mg/kg, *i.p.*, Sigma) for a total of 5 injections administered every other day. Mice were monitored for 30 min, and time spent repeatedly breaking the same photobeam during the 30 min after cocaine injection was normalized to time accumulated during the half hour prior to injection; sessions 1 and 5 were compared by 2-factor repeated measures ANOVA with group and session as factors. Fisher's *post hoc* comparisons were applied when appropriate.

Repeated cocaine exposure increased locomotor activity, reflecting classical psychomotor sensitization [main effect of session $F(1,33) = 21.1$, $p < 0.001$]. Moreover, *all* mice with a history of early-life HA-1077 exposure were more active than control

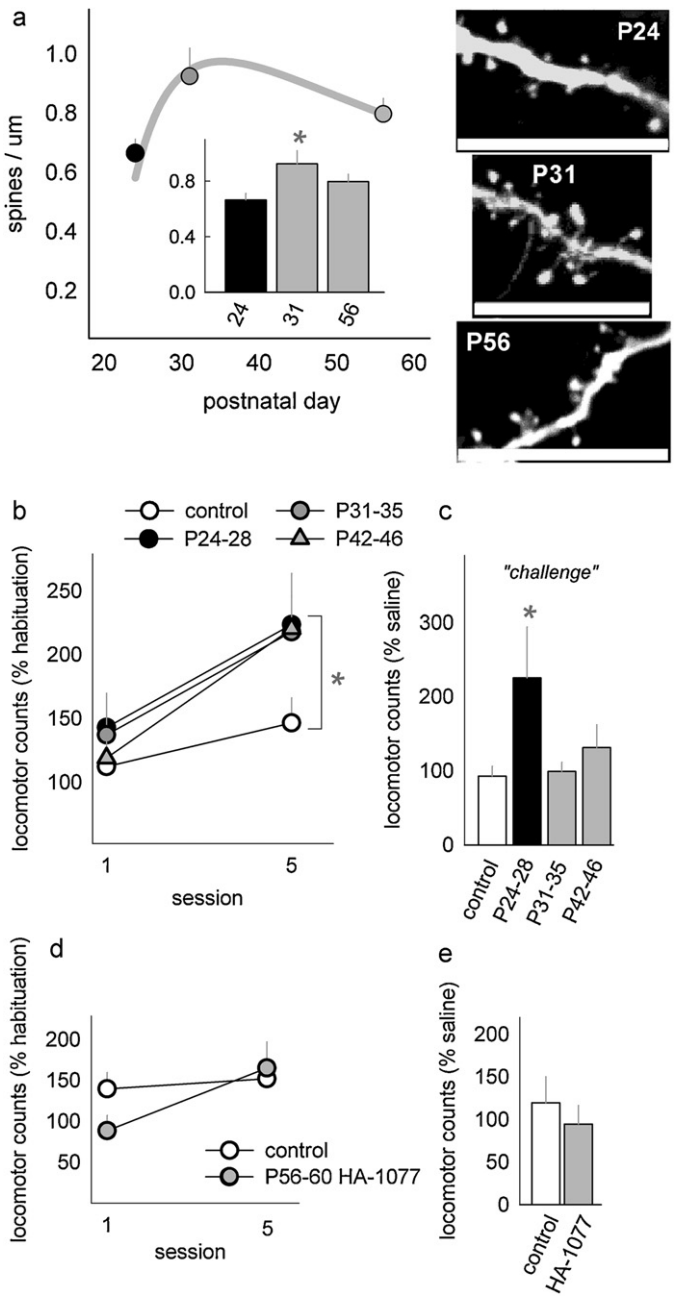

Fig. 1. Rho-kinase (ROCK) inhibition during adolescent dendritic spine maturation confers hyper-sensitivity to cocaine in adulthood. (a) Deep-layer orbitofrontal cortical spine density increases from P24 to P31 and then declines between P31 and P56. Curve fits are shown for illustrative purposes. Inset: Density measures are also shown in bar graph form. Representative spines are shown at right. Scale bar = 5 μm, $n = 18$ neurons (7 mice)/group. (b) Injections of the ROCK inhibitor HA-1077 from P24–28, P31–35, or P42–46 increase psychomotor sensitivity to cocaine *in adulthood*, $n = 6$–9/group. Control groups did not differ and were collapsed. (c) Despite a 2-week washout period, sensitivity remains exaggerated in adult mice exposed to HA-1077 from P24–28. (d) Psychomotor sensitivity to cocaine does not, however, differ in mice pretreated with HA-1077 in adulthood rather than adolescence, $n = 5$–6/group. (e) We also detected no differences after a 2-week washout period. Bars and symbols represent means + SEMs, $^*p < 0.05$ *vs.* control.

mice, indicating heightened sensitivity to cocaine [main effect of HA-1077 $F(3,33) = 3.0$, $p = 0.04$; *post hoc* ps ≤ 0.05] (Fig. 1b).

Following the sensitization procedure, mice were allowed a 2-week washout period, followed by a "challenge" injection. Mice were again habituated to the locomotor chambers for 1 h, administered PBS, monitored for an additional hour, and then administered a final injection of cocaine (10 mg/kg) and monitored