Published in final edited form as:

Behav Pharmacol. 2013 August ; 24(4): 332–336. doi:10.1097/FBP.0b013e328363367b.

Narp expressed in medial prefrontal cortex neurons is not necessary for extinction of heroin self-administration

Ashley M. Blouin, Ph.D.^{1,‡}, Anna L. Stern, B.S.⁴, Sungho Han, B.S.¹, Florence R. Theberge, Ph.D.⁴, Chuansong Wang, Ph.D.², Matthew J. During, M.D., Sc.D.², Jay M. Baraban, M.D., Ph.D.^{1,3}, and Irving M. Reti, M.B.B.S.^{1,3}

¹ Department of Psychiatry and Behavioral Sciences Johns Hopkins School of Medicine, Baltimore, MD, 21205, USA

² Department of Molecular Virology, Immunology and Molecular Genetics, Ohio State University, Columbus, OH, 43210, USA

³ Solomon Snyder Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD, 21205, USA

⁴ NIH/NIDA/IRP, Baltimore, MD, 21224, USA

Abstract

The medial prefrontal cortex (mPFC) plays a key role in extinction learning. Previously, we found that expression of a Narp dominant-negative construct in the mPFC of mice blocked extinction of morphine conditioned place preference. In order to further investigate the role of mPFC Narp in the extinction of drug seeking, we tested whether mPFC Narp is necessary for the extinction of heroin self-administration in rats. Specifically, we injected an adeno-associated viral vector (AAV) expressing a dominant-negative form of Narp (NarpN) into the infralimbic region (IL) of the mPFC of rats and compared lever presses during extinction to those of rats injected with a control virus. In contrast to our previous study, we found that injection of NarpN did not affect extinction of heroin self-administration. Our findings suggest that mPFC Narp is necessary for extinction of opiate seeking in the Pavlovian conditioned place preference paradigm but not in the operant paradigm of drug self-administration.

Keywords

Narp; extinction; heroin; self-administration; addiction; prefrontal cortex

Introduction

Neuronal activity-regulated pentraxin (Narp) is an immediate early gene product that is secreted and binds to AMPA receptors (O'Brien *et al*, 1999). Although Narp knockout (KO) mice acquire instrumental and Pavlovian learning normally, they are deficient in extinction of morphine conditioned place preference (CPP; Crombag *et al*, 2009; Johnson *et al*, 2007). Recently, we determined that lack of Narp in the infralimbic region (IL) of the medial prefrontal cortex (mPFC), a region that plays a crucial role in extinction of drug seeking (LaLumiere *et al*, 2010; Peters *et al*, 2008), mediates this phenotype. We found that viral-mediated expression of a Narp dominant-negative construct in the IL of wild-type mice

[‡]To whom correspondence should be addressed. Ashley M. Blouin Johns Hopkins School of Medicine Ross Research Building Rm 618 720 Rutland Ave Baltimore, MD 21205 Tel: 310-594-3476 Fax: 410-502-3769 ablouin1@jhmi.edu.
The authors report no conflicts of interest.

blocks extinction of morphine CPP while viral-mediated expression of wild-type Narp in the IL of knockout mice rescues extinction of morphine CPP (Blouin *et al*, 2013).

In the current study, we investigated the role of IL Narp in opiate self-administration, since the rodent self-administration model may provide a more salient model of human drug addiction (Brady, 1991; Panlilio and Goldberg, 2007). Specifically, we determined the effect of viral-mediated knockdown of Narp in the IL of rats during extinction of heroin self-administration and hypothesized that this manipulation would block extinction in this paradigm.

Materials and Methods

Subjects

Heroin self-administration was conducted using a total of 32 male Sprague Dawley rats, although 5 were excluded from the study due to failure of catheter patency. All animals were approximately 10 weeks of age at the time of surgery. Rats were kept on a reverse light/dark cycle (lights on 21:00-09:00), and heroin self-administration was performed during the hours of 09:00-16:00. Food and water were available ad libitum in the home cage.

Surgery

Rats were anesthetized with a mixture of sodium pentobarbital + chloral hydrate (60 and 25 mg/kg, respectively, ip). One μ l of either AAV-NarpN or AAV-yellow fluorescent protein (YFP) was injected per side through bilateral guide cannulae (23-gauge, Plastics One, Roanoke, VA), and all injections were made at a 10° angle into the IL mPFC according to the following coordinates +3.0, $\pm$ 1.5, -4.3 (Paxinos G, 2005). AAV-NarpN has been shown to block trafficking of endogenous Narp into spinal and cortical axons in cultures from rat (Johnson *et al*, 2010; O'Brien *et al*, 2002). Viral products were titrated by Dr. During's laboratory and were approximately $1-5 \times 10^{12}$ particles/ml (see Johnson *et al*, 2010 for more details on AAV-NarpN). Immediately following viral injections, rats were implanted with silastic catheters into the jugular vein; the catheters passed subcutaneously to the top of the skull and attached to a modified 22-gauge cannula (Plastics One, Roanoke, VA) that was mounted to the rat's skull with dental cement (Lu *et al*, 2004; Lu *et al*, 2007). Buprenorphine (0.1 mg/kg, s.c.) was administered as an analgesic after surgery (per the requirements of the local ACUC), and the rats were allowed to recover for 5-7 days. During the recovery and training phases, catheters were flushed every 24-48 hours with sterile saline and gentamicin (5 mg/mL, Butler Schein).

Heroin self-administration

5-7 days after viral injection through cannulae and jugular vein catheter implantation, rats were trained to self-administer heroin (Airavaara *et al*, 2011; Theberge *et al*, 2012). For training, rats were placed in operant chambers for 3 hours per day for 14 days. One active lever press activated a pump and resulted in a 0.075mg/kg heroin infusion at a volume of 65 μ L over 2.3 sec (diacetylmorphine HCl, National Institute on Drug Abuse, Baltimore, MD, dissolved in saline) coinciding with a 2.3 sec presentation of a tone-light cue. Each infusion was followed by a 20 sec time out during which active lever presses did not result in infusions. Each chamber also contained an inactive lever, which had no programmed consequences when pressed. Following the training phase, rats remained in home cages for 14 days without exposure to operant chambers to allow for adequate viral expression. During this 14-day withdrawal phase, rats were handled 3-4 times per week. Rats then underwent extinction for 3 hours per day for 14 days; this phase consisted of exposure to the same chambers used during training, but a lever press on the previously active lever resulted in a tone-light cue only and no heroin. Following extinction, 3- hour reinstatement sessions

were administered daily over 6 days. For the first three days, rats were given s.c. priming injections of saline, 0.13 mg/kg heroin, and 0.3 mg/kg heroin on consecutive days. On the subsequent three days, rats were administered i.p. priming injections of water, 1 mg/kg yohimbine (Sigma, St. Louis, MO), and 2 mg/kg yohimbine on consecutive days. Yohimbine is an anxiogenic drug used for stress-induced reinstatement procedures (Nair *et al*, 2011). Infusions during training and responses on both the active and inactive levers were measured during the 3- hour training, extinction and reinstatement sessions.

Immunostaining and quantification of viral injections

Rats were perfused with 4% paraformaldehyde, and mPFC sections were processed for histology. As the NarpN construct contains a C-terminal myc tag, NarpN expression was monitored with a mouse anti-myc primary antibody (1:1000; Invitrogen, Grand Island, NY, USA) and a biotinylated anti-mouse secondary antibody (1:500; Vector) combined with DAB (Vector). YFP fluorescence was visualized directly. The percentage of cells expressing AAV-NarpN in the IL region was then quantified as in Blouin *et al* (2013). To assess the spread of the virus, the percentage of cells expressing viral product in the prelimbic (PrL) region of the mPFC was also quantified.

Statistical Analysis

A total of 14 rats injected with AAV-NarpN and 13 rats injected with AAV-YFP were included in the analysis. Repeated measures ANOVA was used to analyze self-administration time course data for training, extinction and reinstatement. The dependent variables were active and inactive lever presses per 3 hour session for the training, extinction and reinstatement phases. For training, infusions per 3 hour session were also analyzed. Paired t-tests were used to compare viral expression in the IL and PrL regions. Viral expression was correlated with active lever presses per 3 hour reinstatement session using Pearson's correlational analysis.

Results

Rats with IL injections of NarpN or YFP showed normal acquisition of heroin self-administration and did not differ in the number of active lever presses or heroin infusions (Fig 1A and 1B). A Virus Condition (YFP or NarpN) $\times$ Training Day (Day 1-14) repeated measures ANOVA for active lever presses/3hr showed a significant effect of Training Day ($F(13, 325)=3.66, p<0.001$) but no effect of Virus Condition or Training Day $\times$ Virus Condition interaction (p values >0.1). Likewise, a repeated measures ANOVA for infusions/3hr showed only an effect of Training Day ($F(13, 325)=5.86, p<0.001$). Contrary to what we hypothesized, rats with IL injections of NarpN did not differ from those with YFP injections in extinction of heroin self-administration (Fig 1C). A Virus Condition (YFP or NarpN) $\times$ Extinction Day (Day 1-14) repeated measures ANOVA for active lever presses/3hr showed a significant effect of Extinction Day ($F(13, 325)=63.36, p<0.001$) but no effect of Virus Condition or Extinction Day $\times$ Virus Condition interaction (p values >0.1).

For reinstatement with heroin priming injections, a repeated measures ANOVA for active lever presses/3hr showed a significant effect of Dose ($F(2,50)=11.14, p<0.001$) but no significant effects of Virus Condition or Virus Condition $\times$ Dose (Fig 1D). Similarly, for reinstatement with yohimbine, a repeated measures ANOVA for active lever presses/3hr showed a significant effect of Dose ($F(2, 50)=16.00, p<0.001$) but no significant effects of Virus Condition or Virus Condition $\times$ Dose (Fig 1D). No differences were found between the YFP and NarpN injected rats for inactive lever presses during acquisition, extinction or reinstatement.

We found that over 50% of the IL mPFC was infected by AAV-NarpN, and this did not differ from infection by AAV-YFP (Fig 2). The viral expression in the IL mPFC was significantly greater than viral expression which spread to the PrL mPFC (paired t-tests; NarpN: IL=54.29 $\pm$ 8.30% vs PrL=36.43 $\pm$ 4.24%, $p=0.0256$; YFP: IL=51.92 $\pm$ 6.14% vs PrL=21.15 $\pm$ 2.95%, $p<0.0001$). Viral expression did not correlate with active lever presses during the 3hr reinstatement sessions with yohimbine.

Discussion

Previously, we showed that selective expression of Narp in the IL mPFC rescues the Narp KO phenotype and selective knockdown of Narp in the IL mPFC recapitulates the Narp KO phenotype in the morphine CPP paradigm (Blouin *et al*, 2013). In contrast to the key role of IL Narp in the extinction of morphine CPP, it appears to be unnecessary for the extinction of heroin self-administration. This finding may represent a species difference, as mice were used for morphine CPP and rats for heroin self-administration. Thus, it is possible that although Narp is expressed in the IL region of rats, other molecular mechanisms may exist that can compensate for the loss of Narp in the rat although this is not the case in the mouse. A control experiment testing the effect of NarpN on the extinction of opiate-induced CPP in rats is needed to rule out the possibility that our findings are due to a species difference. Since morphine was used in our prior CPP experiments and heroin for the self-administration studies, it also remains possible that Narp is specifically involved in morphine addiction but not heroin addiction. However, both NarpN and YFP viral expression in the PrL and IL regions of the rats in the current study was similar to that of the mice in our previous study (Blouin *et al*, 2013). Thus, our result cannot be attributed to more NarpN in the IL region of the mouse nor to an altered pattern of expression in the mouse compared to the rat.

An alternative explanation for our results is that Narp is selectively involved in the extinction of drug associations present during CPP but not during drug self-administration. Although both paradigms involve drug associations, they differ in many ways. In the CPP paradigm, the association between drug administration and the environment is non-contingent upon the response of the animal (Bardo and Bevins, 2000). However, during self-administration, the animal must execute a response to receive drug (Panlilio *et al*, 2007). Plasticity-associated gene expression patterns have been shown to differ when the association between drug administration and the environment depends on the response of the animal versus when the association is purely Pavlovian (Thomas *et al*, 2003; Thomas and Everitt, 2001). Furthermore, the self-administration paradigm may involve a heightened element of expectancy because the animal expects the drug immediately after the lever press. Expectancy for a reinforcer, especially following an animal's own behavior, has been shown to modulate the physiological response to that reinforcer (Garrud *et al*, 1981; Jacobs *et al*, 2003; Weise-Kelly and Siegel, 2001). Thus, the absence of expected reward during extinction of self-administration may be able to engage mechanisms capable of eliciting extinction that are not dependent on Narp released from mPFC neurons.

In summary, we find that Narp knockdown in the IL mPFC of the rat does not affect extinction of heroin self-administration. It is possible that our finding represents a species difference with Narp being necessary for extinction in the mouse but not in the rat. However, it is also likely that our previous finding that mPFC Narp mediates extinction of morphine CPP may apply to the passive associations made during Pavlovian processes. During extinction of the operant paradigm of drug self-administration, the absence of expected reward following the animal's response may override the need for Narp. Thus, Narp in the mPFC may not be needed for all types of extinction and may play a selective

role in mediating drug craving elicited by exposure to environmental cues associated with drug reward.

Acknowledgments

The behavioral part of this paper was performed at the IRP-NIDA in the laboratory of Dr. Yavin Shaham. The authors would like to thank Dr. Yavin Shaham for his assistance in the design and analysis of this study, Dr. Peter Holland for helpful comments on the manuscript, and Drs. Alexander Johnson and Gregory Michealson for statistical advice. The Narp antibody was provided by Dr. Paul Worley. This work was funded by RO1 DA016303, NIDA R25DA21630-3 and funds to the intramural research program of NIDA.

This work was funded by RO1 DA016303, NIDA R25DA21630-3 and funds to the intramural research program of NIDA.

References

- Airavaara M, Pickens CL, Stern AL, Wihbey KA, Harvey BK, Bossert JM, et al. Endogenous GDNF in ventral tegmental area and nucleus accumbens does not play a role in the incubation of heroin craving. *Addict Biol.* 2011; 16(2):261–272. [PubMed: 21182575]
- Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)*. 2000; 153(1):31–43. [PubMed: 11255927]
- Blouin AM, Han S, Pearce AM, Cheng K, Lee JJ, Johnson AW, et al. Role of medial prefrontal cortex Narp in the extinction of morphine conditioned place preference. *Learn Mem.* 2013; 20(2):75–79. [PubMed: 23322555]
- Brady JV. Animal models for assessing drugs of abuse. *Neurosci Biobehav Rev.* 1991; 15(1):35–43. [PubMed: 2052196]
- Crombag HS, Dickson M, Dinenna M, Johnson AW, Perin MS, Holland PC, et al. Narp deletion blocks extinction of morphine place preference conditioning. *Neuropsychopharmacology.* 2009; 34(4):857–866. [PubMed: 18536700]
- Garrud P, Goodall G, Mackintosh NJ. Overshadowing of a stimulus-reinforcer association by an instrumental response. *The Quarterly Journal of Experimental Psychology Section B: Comparative and Physiological Psychology.* 1981; 33(2):123–135.
- Jacobs EH, Smit AB, de Vries TJ, Schoffelemeier AN. Neuroadaptive effects of active versus passive drug administration in addiction research. *Trends Pharmacol Sci.* 2003; 24(11):566–573. [PubMed: 14607079]
- Johnson AW, Crombag HS, Takamiya K, Baraban JM, Holland PC, Haganir RL, et al. A selective role for neuronal activity regulated pentraxin in the processing of sensory-specific incentive value. *J Neurosci.* 2007; 27(49):13430–13435. [PubMed: 18057201]
- Johnson AW, Han S, Blouin AM, Saini J, Worley PF, During MJ, et al. Localized disruption of Narp in medial prefrontal cortex blocks reinforcer devaluation performance. *Learn Mem.* 2010; 17(12):620–626. [PubMed: 21127001]
- LaLumiere RT, Niehoff KE, Kalivas PW. The infralimbic cortex regulates the consolidation of extinction after cocaine self-administration. *Learn Mem.* 2010; 17(4):168–175. [PubMed: 20332188]
- Lu L, Grimm JW, Dempsey J, Shaham Y. Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. *Psychopharmacology (Berl)*. 2004; 176(1):101–108. [PubMed: 15071719]
- Lu L, Uejima JL, Gray SM, Bossert JM, Shaham Y. Systemic and central amygdala injections of the mGluR(2/3) agonist LY379268 attenuate the expression of incubation of cocaine craving. *Biol Psychiatry.* 2007; 61(5):591–598. [PubMed: 16893525]
- Nair SG, Navarre BM, Cifani C, Pickens CL, Bossert JM, Shaham Y. Role of dorsal medial prefrontal cortex dopamine D1-family receptors in relapse to high-fat food seeking induced by the anxiogenic drug yohimbine. *Neuropsychopharmacology.* 2011; 36(2):497–510. [PubMed: 20962767]

- O'Brien R, Xu D, Mi R, Tang X, Hopf C, Worley P. Synaptically targeted Narp plays an essential role in the aggregation of AMPA receptors at excitatory synapses in cultured spinal neurons. *J Neurosci*. 2002; 22(11):4487–4498. [PubMed: 12040056]
- O'Brien RJ, Xu D, Petralia RS, Steward O, Huganir RL, Worley P. Synaptic clustering of AMPA receptors by the extracellular immediate-early gene product Narp. *Neuron*. 1999; 23(2):309–323. [PubMed: 10399937]
- Panlilio LV, Goldberg SR. Self-administration of drugs in animals and humans as a model and an investigative tool. *Addiction*. 2007; 102(12):1863–1870. [PubMed: 18031422]
- Paxinos, GWC. The rat brain in stereotaxic coordinates. 5th. Elsevier Academic Press; San Diego, CA: 2005.
- Peters J, LaLumiere RT, Kalivas PW. Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *J Neurosci*. 2008; 28(23):6046–6053. [PubMed: 18524910]
- Theberge FR, Pickens CL, Goldart E, Fanous S, Hope BT, Liu QR, et al. Association of time-dependent changes in mu opioid receptor mRNA, but not BDNF, TrkB, or MeCP2 mRNA and protein expression in the rat nucleus accumbens with incubation of heroin craving. *Psychopharmacology (Berl)*. 2012; 224(4):559–571. [PubMed: 22790874]
- Thomas KL, Arroyo M, Everitt BJ. Induction of the learning and plasticity-associated gene Zif268 following exposure to a discrete cocaine-associated stimulus. *Eur J Neurosci*. 2003; 17(9):1964–1972. [PubMed: 12752796]
- Thomas KL, Everitt BJ. Limbic-cortical-ventral striatal activation during retrieval of a discrete cocaine-associated stimulus: a cellular imaging study with gamma protein kinase C expression. *J Neurosci*. 2001; 21(7):2526–2535. [PubMed: 11264326]
- Weise-Kelly L, Siegel S. Self-administration cues as signals: drug self-administration and tolerance. *J Exp Psychol Anim Behav Process*. 2001; 27(2):125–136. [PubMed: 11296488]

Fig 1. AAV-NarpN injected into IL mPFC of rats does not block extinction of heroin self-administration. **A**, Active lever presses per 3 hour training session for all 14 days of training. **B**, Heroin infusions per 3 hour session for all 14 days of training. **C**, Active lever presses per 3 hour extinction session for all 14 days of extinction. **D**, Active lever presses per 3 hour reinstatement session for 6 consecutive days of reinstatement

Fig 2. Viral injection into the IL mPFC of rats. **A**, Location of viral injections shown in B-C. **B**, AAV-YFP visualized directly. **C**, AAV-NarpN visualized by myc immunostaining. **D**, Quantification of viral expression in the IL.