

## Rationale

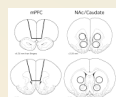
- Previously, we demonstrated time-of-day variation in cocaine sensitization [1], and revealed that day/night differences in cocaine-conditioned place preference rely partially on the SCN and partially on other clock-containing brain regions [2].
- The promoters for the dopamine transporter (DAT) and tyrosine hydroxylase (TH) genes are capable of binding clock proteins [3,4], which are transcription factors expressed in a circadian fashion [5]. Time-of-day differences in cocaine-seeking behavior may thus be influenced by daily variation in DAT and TH. However, it is not known if the expression of DAT and TH changes with the time of day, or if the SCN influences any variation in DAT or TH. Another possibility is that time-of-day variation in cocaine-induced behaviors is mediated by altered function of the DAT protein, and this was also explored in the present study.

## Hypotheses:

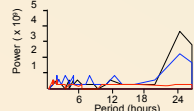
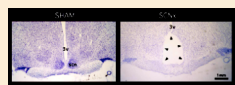
- Expression of DAT and TH in the prefrontal cortex, nucleus accumbens, and caudate varies with time of day, and this variation depends on an intact SCN.
- Rate of dopamine clearance in the prefrontal cortex and nucleus accumbens varies with time of day.

## Methods

- Rats were housed under a 12 hr:12 hr light:dark cycle (ZT0 = Zeitgeber time 0 [0600], lights on; ZT12 = lights off [1800]).
- The SCN was lesioned by passing .034 mA for 10 seconds.
- Bilateral tissue samples (see Figure at right) from the prefrontal cortex, nucleus accumbens, and caudate nucleus were collected 2 wks later at ZT4 or ZT20.
- DAT western blotting: 1:1000 rat anti-DAT, then 1:3000 rabbit anti-rat IgG. TH western blotting: 1:2000 rabbit anti-TH, then 1:3000 goat anti-rabbit IgG. Bands were visualized with luminescence and quantified using ImageJ (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2006).
- Dopamine clearance in prefrontal cortex and nucleus accumbens was measured in tissue collected from surgically naive rats using rotating disc electrode voltammetry [6], after addition of 1  $\mu$ M dopamine. Brains were collected at ZT0, ZT4, ZT8, ZT12, ZT16, and ZT20.

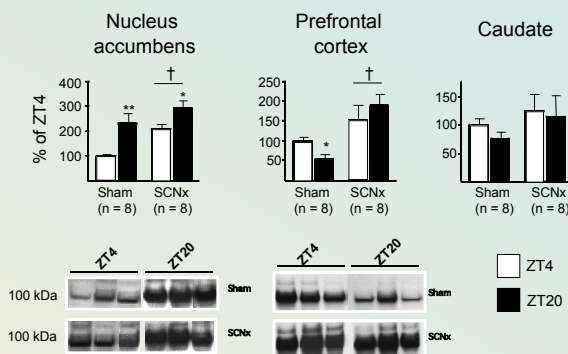


## Confirmation of SCN lesion



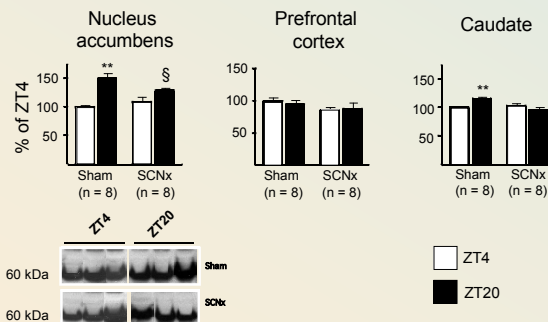
**Left:** Thionin-stained sections at the level of the SCN. **Right:** Fast Fourier transformed locomotor activity from representative Sham (black), SCNx (red), and incomplete SCNx (blue). SCNx rat expresses no 24 hr rhythm.

## Dopamine transporter protein



**Time of day (ZT) affects DAT protein levels in sham and SCNx rats.** Data are expressed as percent of ZT4 sham rats, and are means  $\pm$  SEM. \*  $p < 0.05$  compared to ZT4 within treatment group. \*\*  $p < 0.01$  within treatment group. †  $p < 0.05$  compared to shams.

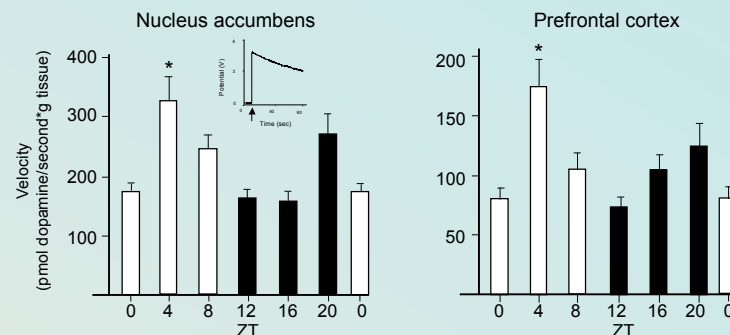
## Tyrosine hydroxylase protein



**Time of day (ZT) affects TH protein levels in sham and SCNx rats.** Data are expressed as percent of ZT4 sham rats, and are means  $\pm$  SEM. \*\*  $p < 0.01$  compared to ZT4 within treatment group. (§)  $p = 0.06$  compared to ZT4 within treatment group.

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## Dopamine transporter function



**Time of day (ZT) affects dopamine clearance in NAc (left) and PFC (right).** Data are means  $\pm$  SEM. \*  $p < 0.05$  compared to ZT0 (N=5-7/ZT). White bars=daytime, dark bars=nighttime. ZT0 is replotted. † Inset: sample voltammetry trace from nucleus accumbens following addition of 1  $\mu$ M dopamine (arrow).

## Conclusions

- DAT protein:**
  - Nucleus accumbens = increased at night, SCN-independent
  - Prefrontal cortex = decreased at night, SCN-dependent
  - Caudate = no changes
- TH protein:**
  - Nucleus accumbens = increased at night, partly SCN-dependent
  - Prefrontal cortex = no changes
  - Caudate = small increase at night, SCN-dependent
- DAT function:**
  - Nucleus accumbens = Diurnal variation with highest DA clearance rate at ZT4
  - Prefrontal cortex = Diurnal variation with highest clearance rate at ZT4

Discrepancies between DAT protein levels and DAT function in the nucleus accumbens at ZT4 compared with ZT20 remain to be resolved, and may be related to membrane trafficking or kinetic activity of the DAT.

## References

- Sleipness EP, Sorg BA, Jansen HT. Time of day alters long-term sensitization to cocaine in rats. *Brain Res* (2005);1065(1-2):132-7.
- Sleipness EP, Sorg BA, Jansen HT. Both time of day and destruction of the suprachiasmatic nucleus (SCN) alter cocaine-conditioned place preference (CPP) behavior in rats. *Soc for Neurosci Abstr* (2005); 341:15.
- Karawai T, Kawakami H, Yamamura Y, Nakamura S. Structure and organization of the gene encoding human dopamine transporter *Gene* (1997); 195(1):11-8.
- Okamura H. Integration of mammalian circadian clock signals: from molecule to behavior. *J Endocrinol* (2003); 177(1):3-6.
- Yoon SO, Chikarashi DM. Isolation of two E-box binding factors that interact with the rat tyrosine hydroxylase enhancer. *J Biol Chem* (1994); 269(28):16453-62.
- Waymerr HK, Schenk JO, Sorg BA. Characterization of extracellular dopamine clearance in the medial prefrontal cortex: role of monoamine uptake and monoamine oxidase inhibition. *J Neurosci* (2002); 22(15):6135-42.