HIV-1 Tat protein: sufficient to cause HIV-Associated Neurological Deficits (HAND)?

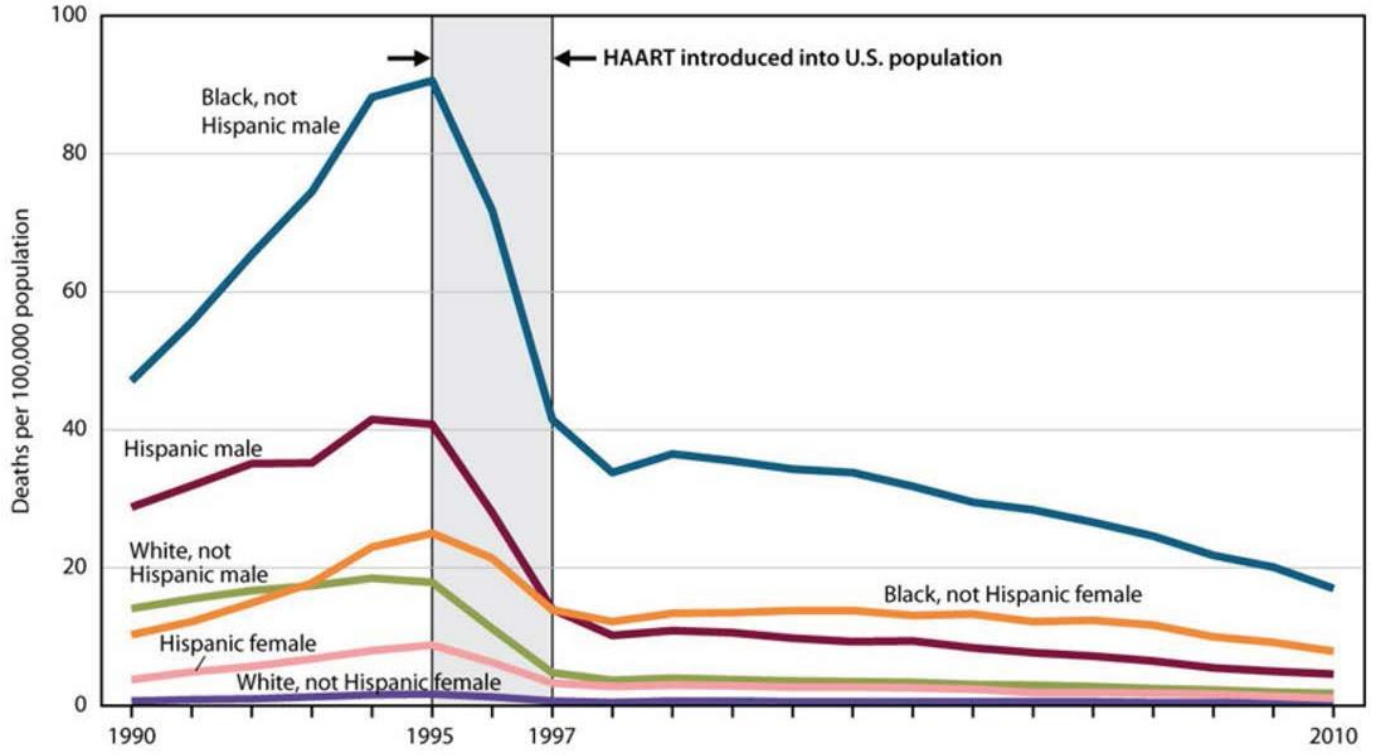
Jay P. McLaughlin

Department of Pharmacodynamics,

University of Florida

Background: Human Immunodeficiency Virus (HIV): changing death rates for all ages suggest transition

- Retrovirus infection responsible for acquired immunodeficiency syndrome (AIDS)
  - Progressive failure of immune system allows life-threatening infections, cancer
- Therapeutics now available have improved prognosis and lifespan



NOTE: HAART is highly active antiretroviral therapy.
SOURCE: CDC/NCHS, Health, United States, 2013, Figure 24. Data from the National Vital Statistics System.

Background: Psychiatric comorbidities remain common among HIV patients

- Even with ART, the prevalence of HIV-associated neurocognitive disorders (HAND) is high (~ 50% in the U.S.) (Heaton et al., 2011)

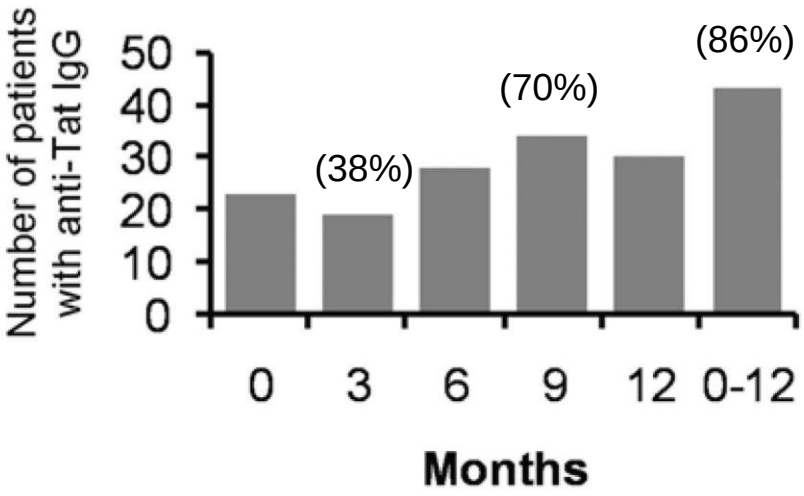
Sakamoto et al., www.slideshare.net/UCSDCFAR/test-forhivassocdementiainindia

- Additional comorbid psychiatric disorders significantly impair quality of life:
  - Anxiety
  - Major Depression
  - Post-Traumatic Stress Disorder
  - Increased substance abuse: alcohol, cocaine, opioids

Background: Persistence of HIV biological factors

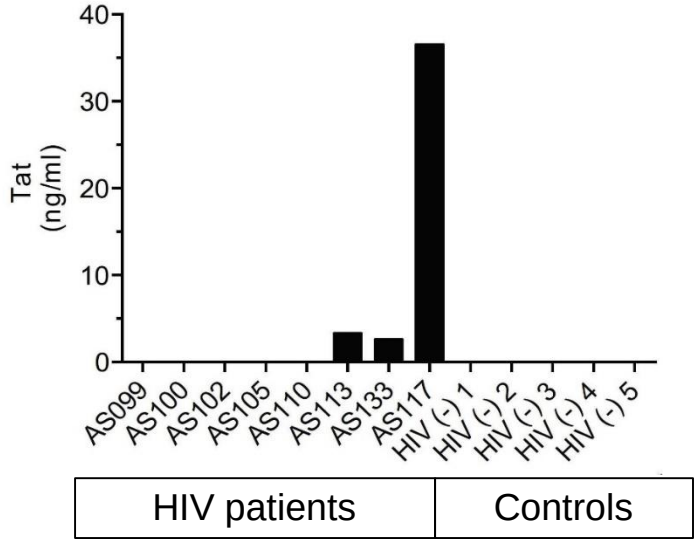
- Although HAART suppresses HIV infection to undetectable levels, it doesn't eliminate latent HIV reservoirs (such as thought to be in CNS)
  - Virus continues to produce and secrete inflammatory cytokines and HIV Transactivator of Transcription (Tat) protein
- Tat: HIV regulatory protein: vastly increases transcription of HIV genes.
  - Secreted intact and functional from infected cells; detected in brain and CSF of HAD patients.

Serum Tat+ (50 HIV Subjects tested quarterly)



Mediouni et al., Infect. Disord. Drug Targets 12:81, 2012

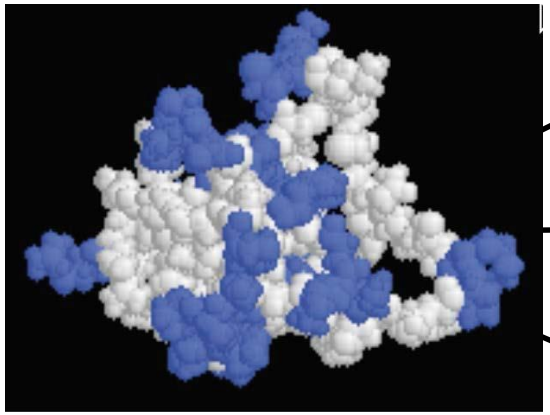
CSF [Tat] in 3 of 8 HIV subjects tested once



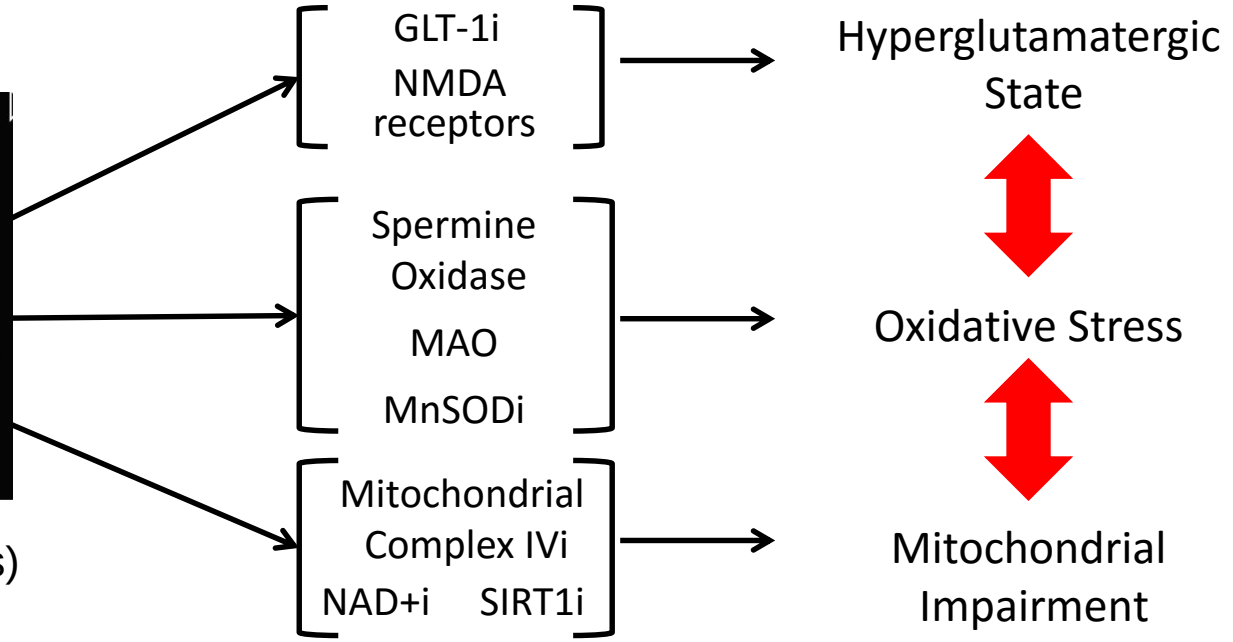
Johnson et al., PNAS 110:13588, 2013

Background: HIV-1 Tat induces a wide range of deleterious biological effects

- Tat protein is neurotoxic; with even transient exposures detrimental



(No known cellular homologs)

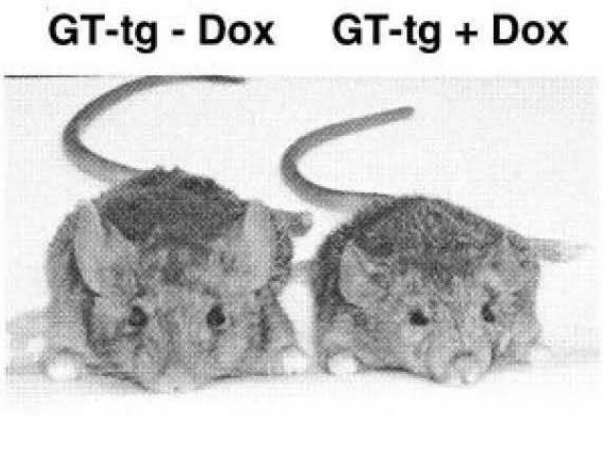


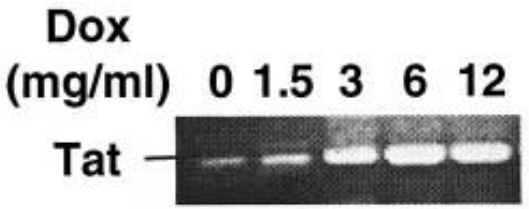
- Tat mediated neurophysiological effects could lead to HIV cognitive dysfunction:
  - Exposure to Tat protein promotes loss of synapses and neuronal death
  - Tat *in vitro* suppresses LTP in hippocampal culture (Behnisch et al., 2004)

- Psychiatric effects unclear, but minimal behavioral evidence implicates Tat:
  - Tat point mutation (Clade C) linked to HAD prevalence and neuron toxicity
  - Injecting Tat: ↑ deficits in working memory in 8-arm maze (Li et al., 2004)
  - Injecting Tat: ↑ depression-like behavior in mouse (Lawson et al., 2011)

Model: Inducible Tat expression with the GT-tg bigenic mouse

- To better understand the role of Tat in neuropathogenesis, dysfunction and behavioral disorders, need transgenic mouse
- Traditional "knock-in" Tat mice show tumors, poor viability (Corallini et al., 1993)
- New model: GT-tg transgenic mouse (Kim et al., Am J. Pathol. 162: 1693; 2003)
  - Gene for Tat protein (from Clade B) is <u>inducible</u> and <u>brain-selective</u>
    - Glial, GFAP-linked Tet-on system conditionally activates Tat synthesis
    - Majority of Tat expression triggered by Doxycycline (Dox)
    - Expression conditional, and only in GFAP-containing astrocytes
  - Expression of Tat produces histopathology as seen with NeuroAIDS
  - Avoids mechanical injury, confounds from direct injection of Tat to brain

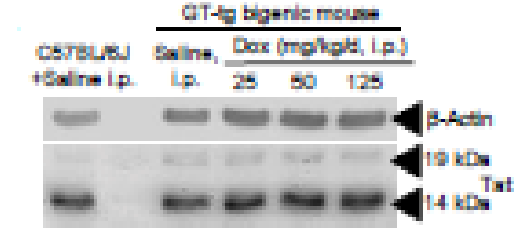


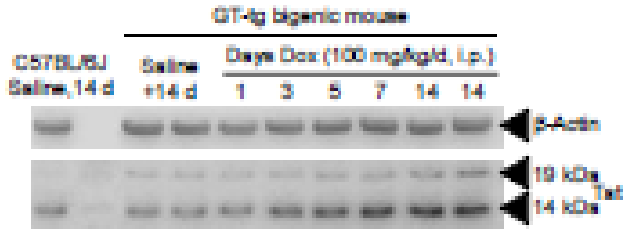


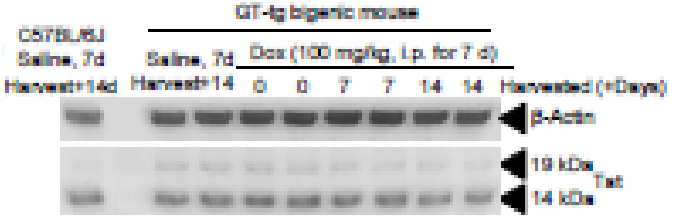
Dox (mg/ml) in drinking water for 8 days (Kim et al., 2003)

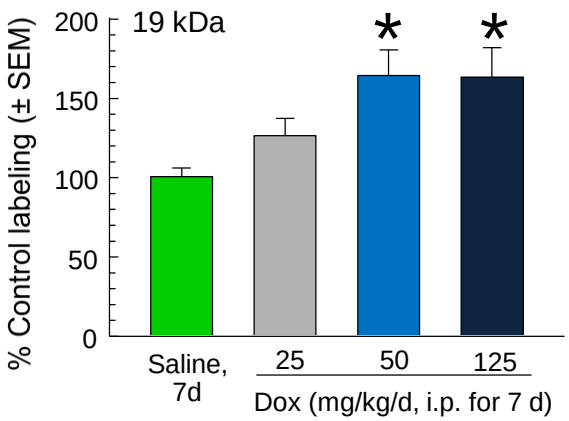
Western blot characterization of doxycycline-mediated Tat expression in the GT-tg mouse brain

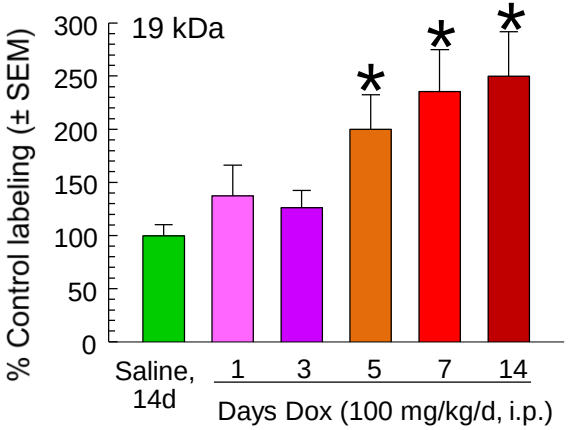
- Limitation: present antibodies for Tat uniformly terrible.
- Results with Abcam polyclonal ab43014, lot #904506 (1:2000) (Carey *et al*., Behav Brain Res, **229:** 2012).

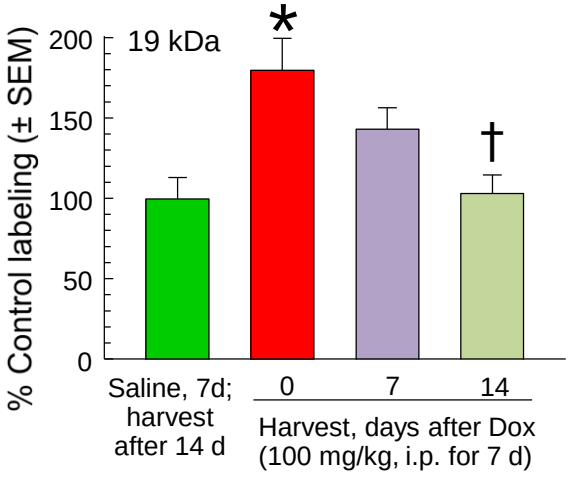






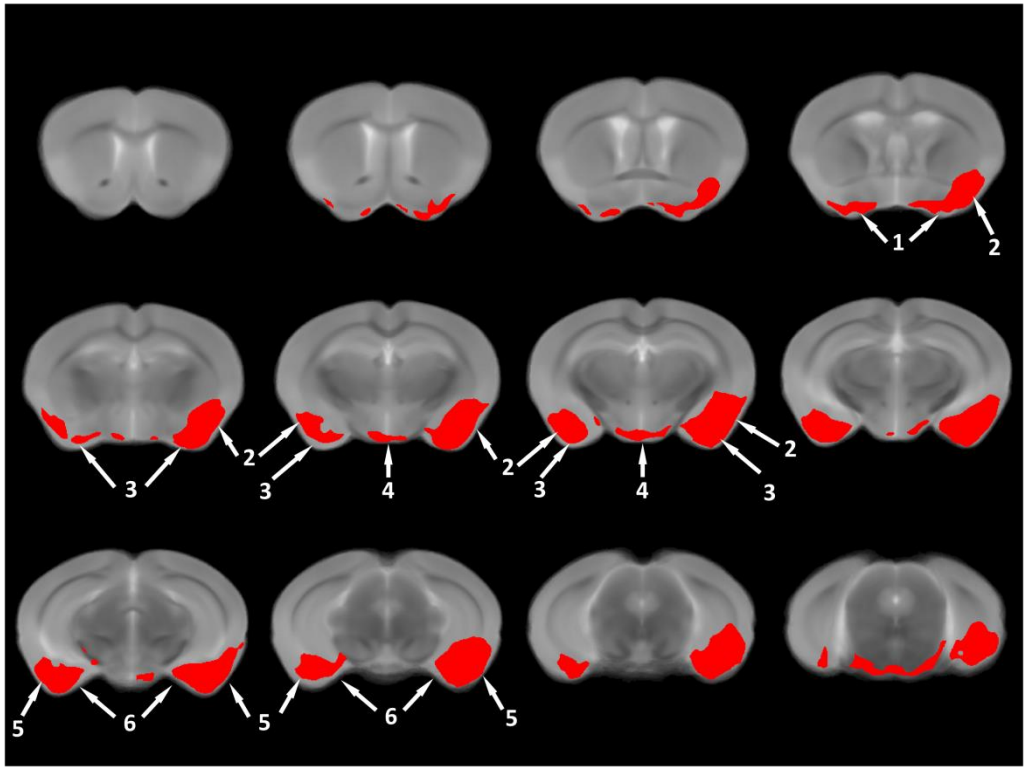


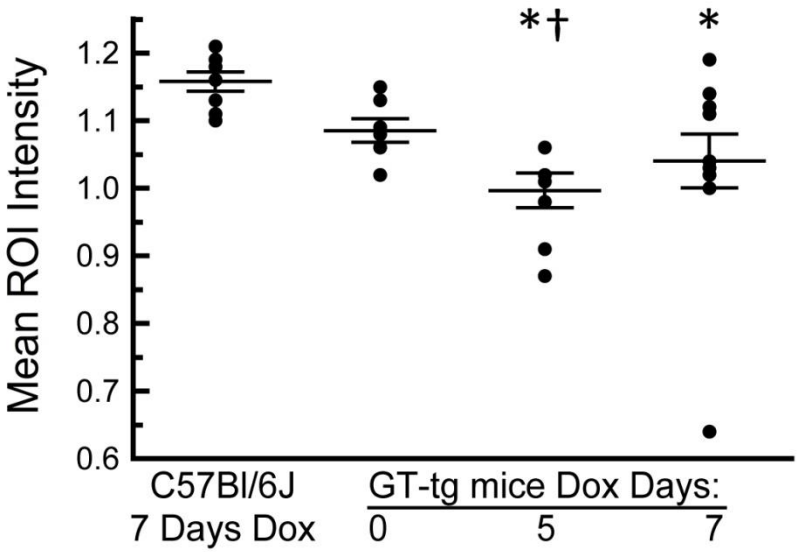




*Paris et al, Psychopharmacology (Berl), **231:** 2014*

Five days' exposure to Tat results in GT-tg mouse brain gray matter density reductions: *ex vivo* MRI

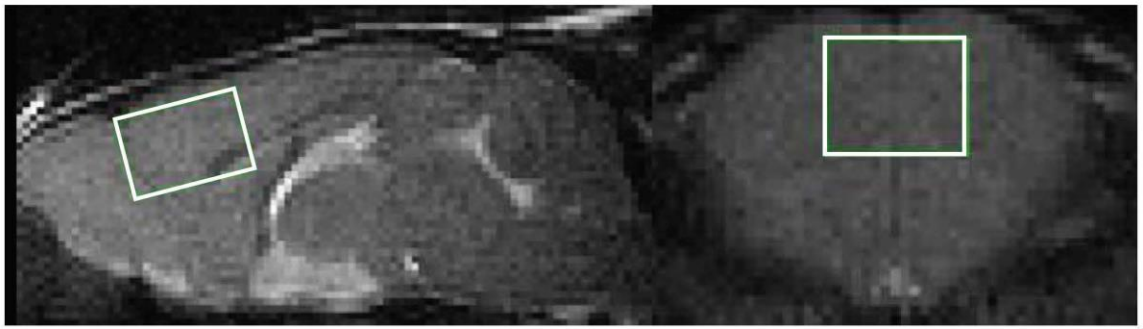




Regions of significant loss:
1) sublenticular extended amygdala, 2) piriform cortex,
3) amygdala, 4) hypothalamus, 5) peri-/entorhinal cortex,
6) amygdala-hippocampal area

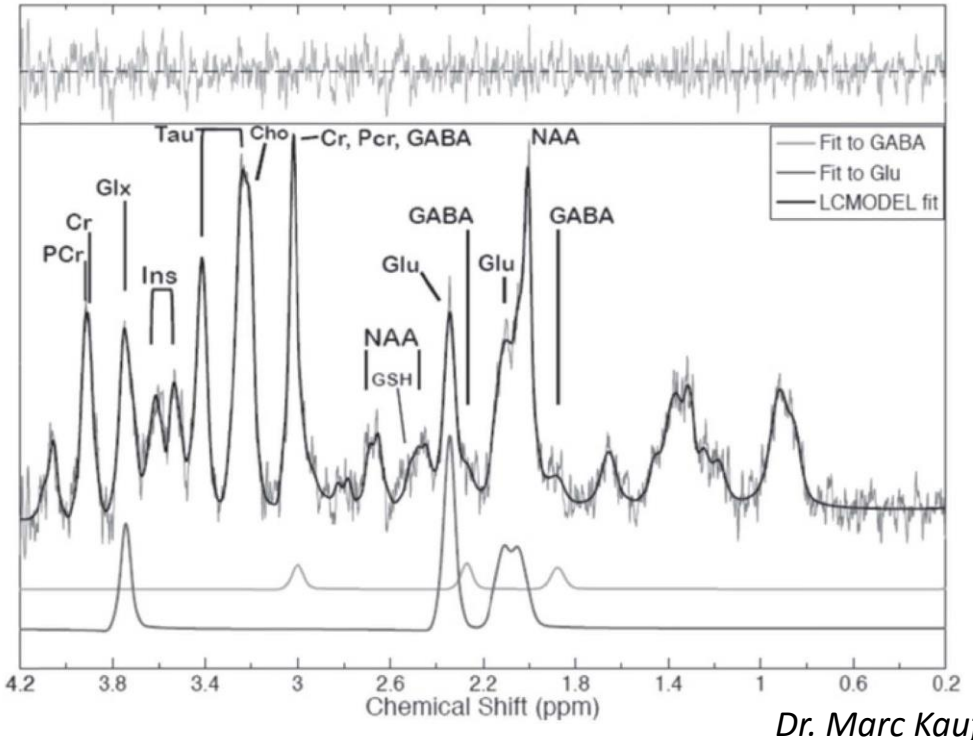
Carey et al, Prog Neuropsychopharmacol Biol Psychiatry, ***43:*** *2013*

Initial Magnetic Resonance Spectroscopy studies suggest Tat-induced neurochemical abnormalities in brains of GT-tg mice

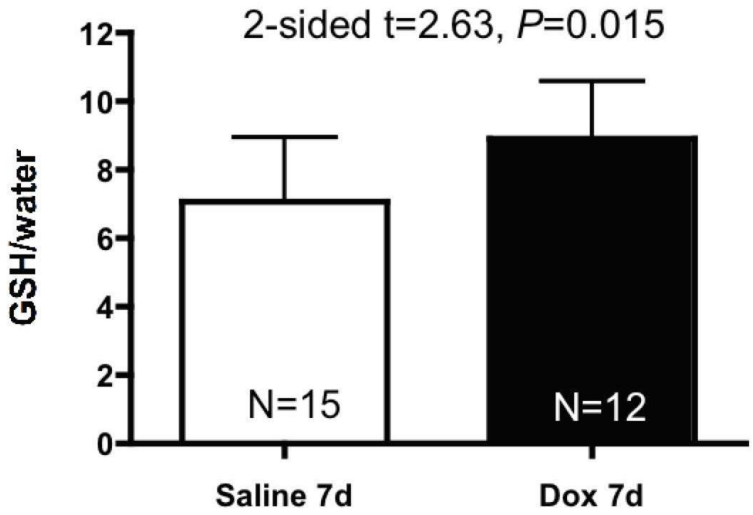


Medial Frontal Cortex,
GT-tg mice

9.4T Proton Magnetic Resonance Spectroscopy



7-d exposure to Tat protein increases medial frontal cortex glutathione (GSH)



Dr. Marc Kaufman, Translational Imaging Laboratory, McLean Hospital

Hypothesis

Global Hypothesis:

Expression of HIV-1 Tat protein in brain is sufficient to produce the neuropsychiatric effects associated with HIV-1 infection

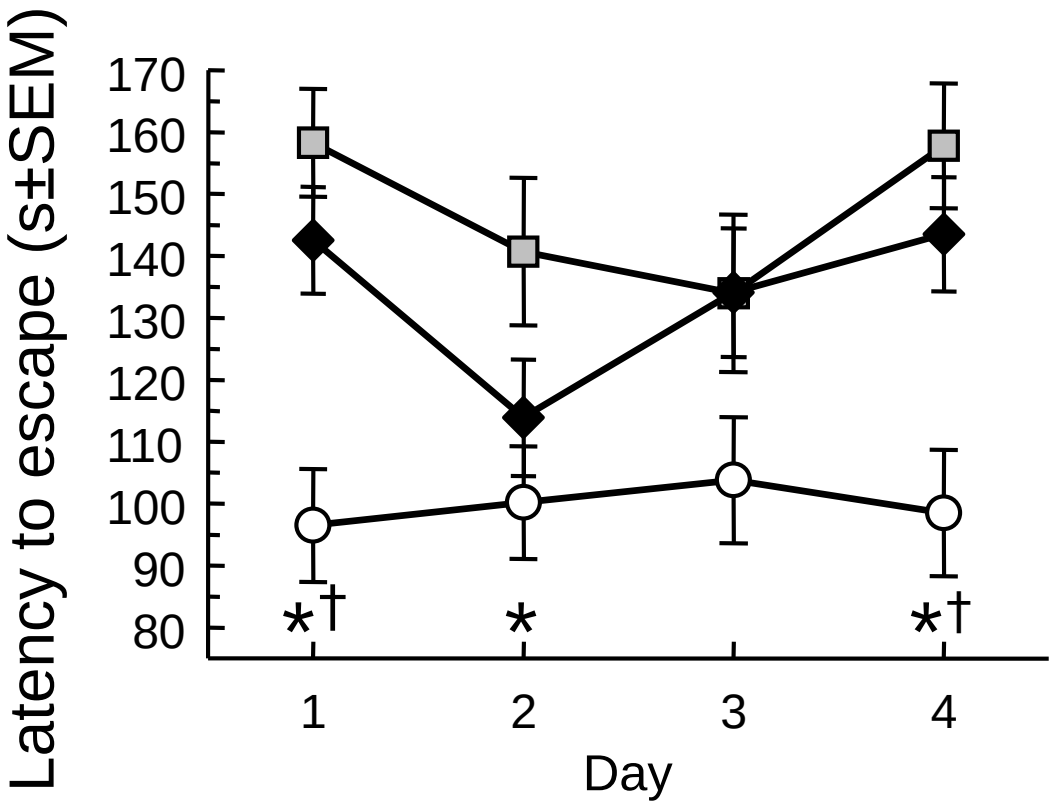
Specific studies:

- Study I: The expression of Tat in brain is sufficient to impair learning and memory performance in the (A) Barnes maze and (B) novel object recognition assays, as well as cognition in (C) the pre-pulse inhibition assay.
- Study II: The expression of Tat in brain is sufficient to induce increases in (A) anxiety- and (B) depression-like behaviors.
- Study III: Tat protein potentiates the psychostimulant and rewarding effects of the reinforcing substances such as morphine in the conditioned place preference (CPP) assay and two-bottle choice assay

Study I: Experiment 1 Methods: The Barnes Maze

- Tests: spatial learning and memory
- Goal: Locate escape box under one of 40 hole using distal cues to navigate
- Motivation: bright open arena & static noise
- Testing schedule
  - Days 1-4: Acquisition, two 3-min trials/day, 15-min ITI
  - Day 4: Probe trial, 90 s
  - Day 5: Reversal learning, 4 trials
- Measures
  - Latency
  - Errors
  - Probe trial success

Study IA: Tat-induced GT-tg mice demonstrated longer latencies to find the escape hole than uninduced GT-tg littermates



GT-tg bigenic mice
- Uninduced
- 5 d 100 mg/kg Dox
- 7 d 100 mg/kg Dox

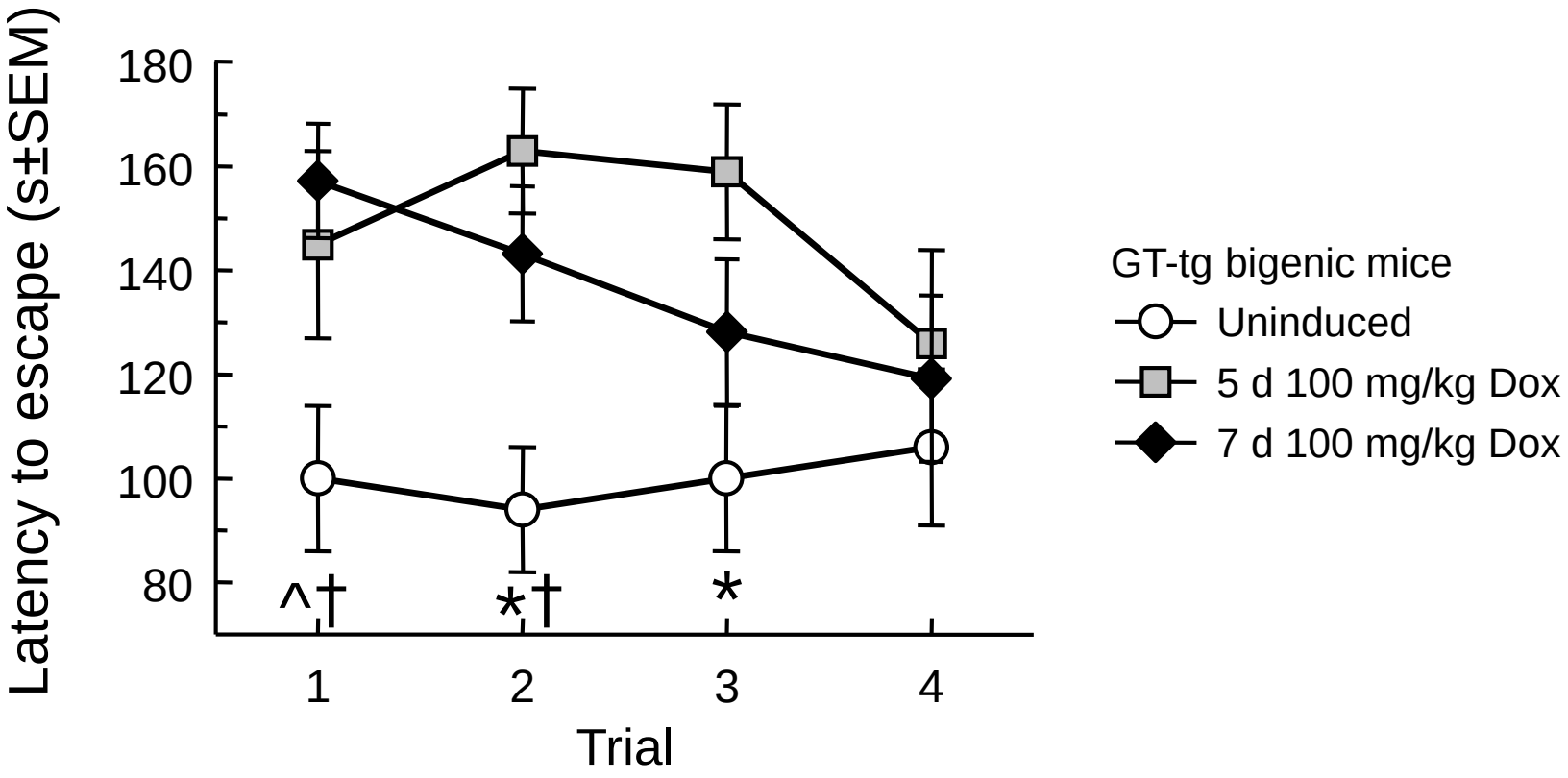
Note: C57Bl/6J show strain Differences from uninduced GT-tg!

Tat-induced GT-tg mice also:

- Committed more errors during acquisition
- Showed significant deficits in finding the escape location during probe trial

Carey et al, Behav Brain Res, ***229:****2012*

Study IA: Tat-induced mice required more trials to learn the new escape location during a reversal learning task



Carey et al, Behav Brain Res, ***229:****2012*

Study I: Experiment 2 Methods: The Novel Object Recognition Assay

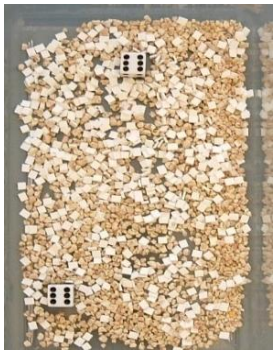
Familiarization/Acquisition Phases 10 min/each

Testing Phase 10 min

Object A

Object B

10 min



10 min

Phase 1
2 Dice

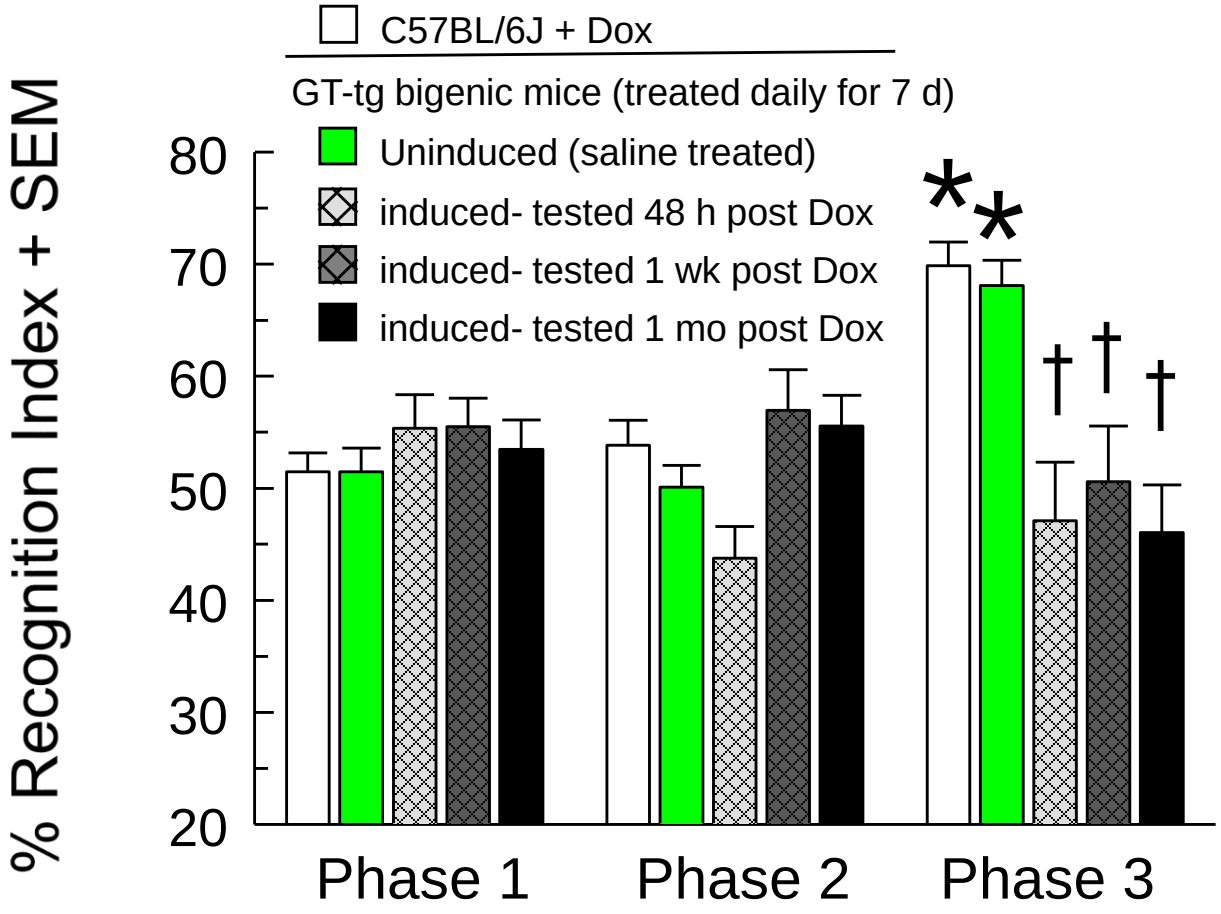
Phase 2
2 Dice

Phase 3
Object B = marble

$$\%\ \text{Recognition Index} = \frac{\text{time spent on object B}}{\text{time spent on object A + B}}$$

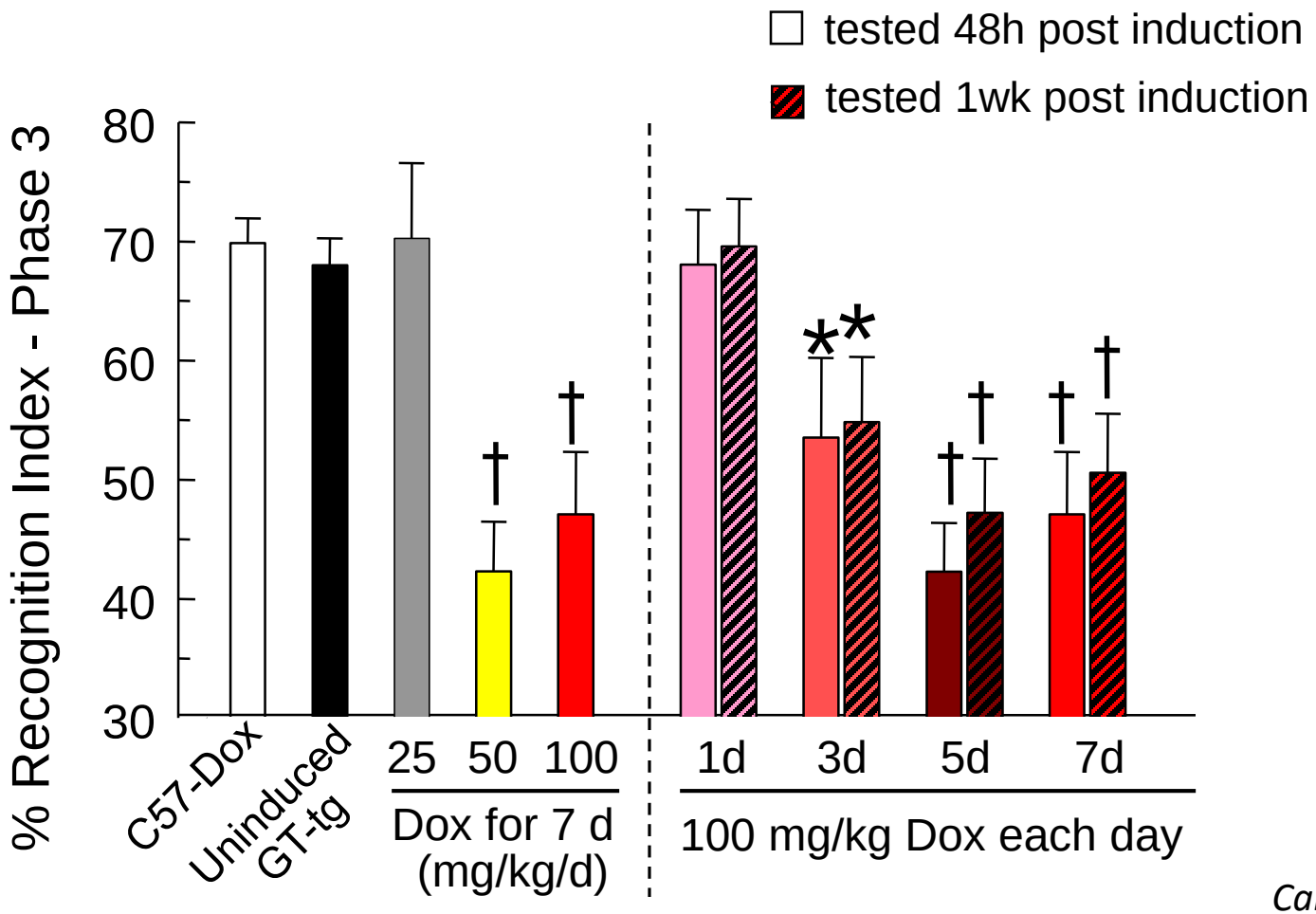
Methods from Carey et al., J Neuroscience 2009

Study IB: Tat-induced impairment of novel object recognition is long-lasting



Carey et al, Behav Brain Res, ***229:****2012*

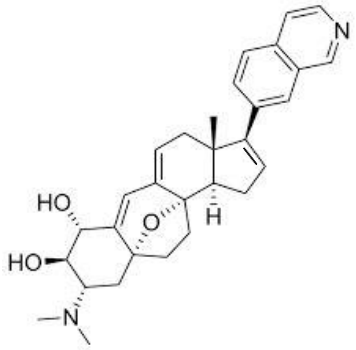
Study IB: Tat-induced impairment of novel object recognition is dependent on the dose of Dox administered and duration of exposure



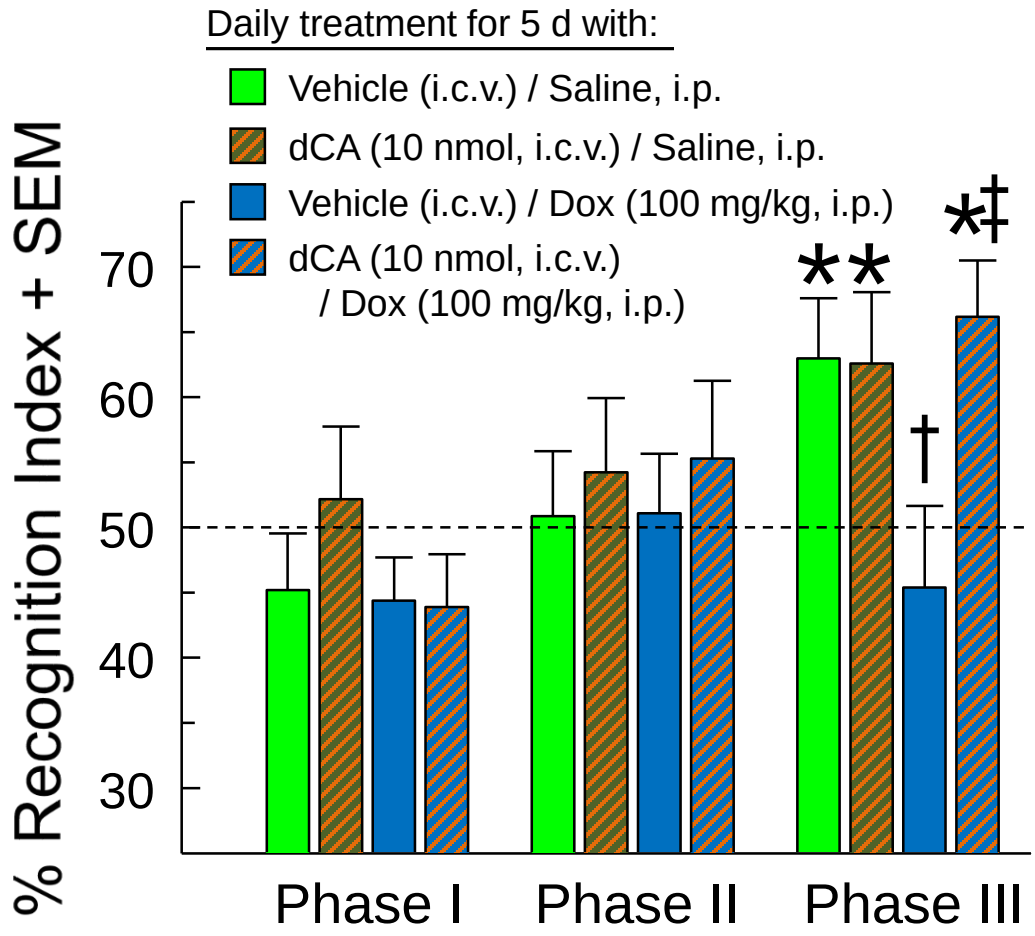
Carey et al, Behav Brain Res, ***229:*** *2012*

Study IB: Prevention of Tat-induced novel object recognition deficits by co-administration of the Tat inhibitor, didehydro-Cortistatin A (dCA)

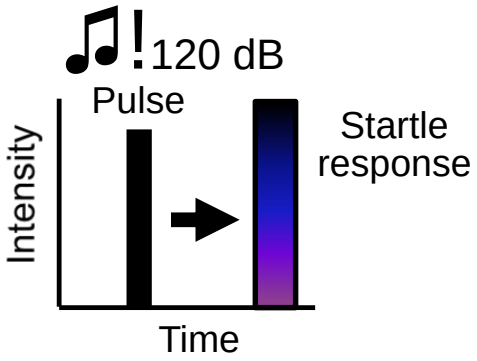
Didehydro-Cortistatin A (dCA)

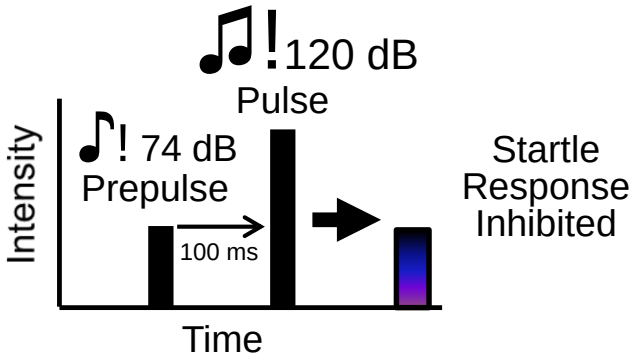


Mousseau et al,
Cell Host & Microbe ***12:****2012*

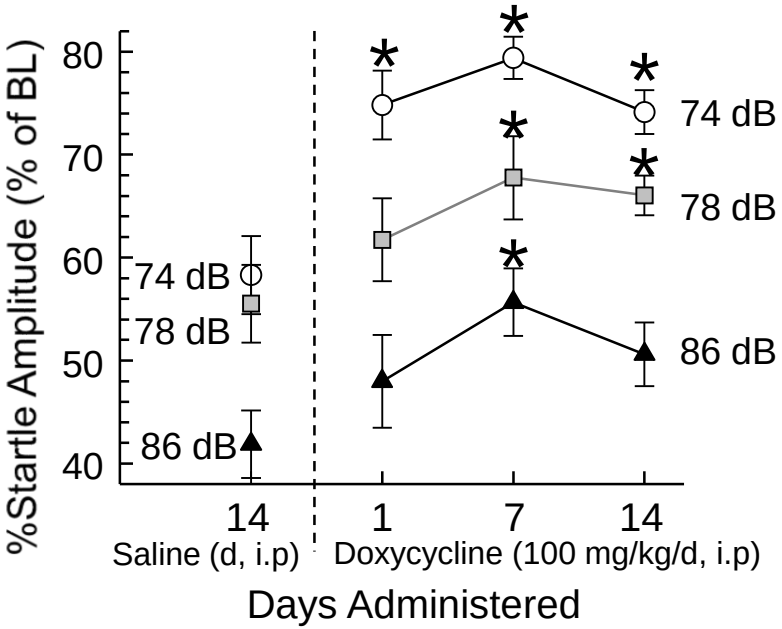


Study 1C: Exposure to Tat protein impairs prepulse inhibition of the acoustic startle response in GT-tg bigenic mice





Pretreatment: Indomethacin (10 mg/kg/d, i.p.)

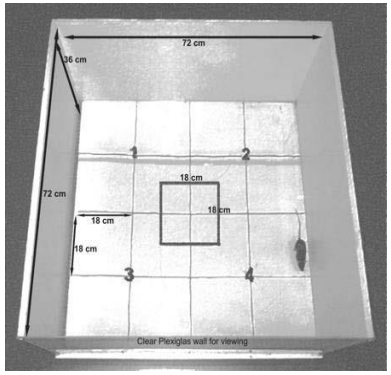


Paris et al., Behav Brain Res, ***291:*** *2015*

Study I: Summary findings

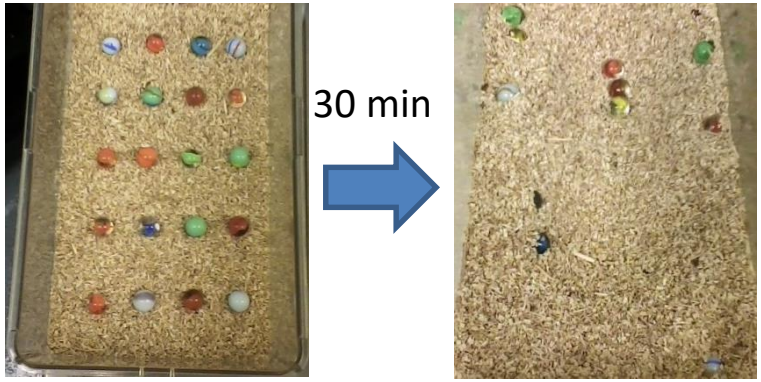
- Mice expressing Tat protein show deficits in spatial learning and memory performance in the Barnes maze
- Mice expressing Tat protein demonstrate perseveration in learned spatial responses.
- Mice expressing Tat protein demonstrate long-lasting impairment of novel object recognition
  - Effect reversed by daily treatment with Tat inhibitor, dCA
- Exposure to Tat protein impaired paired-pulse inhibition in a exposure dependent manner, suggesting impaired cognition
  - Impairment reversed with indomethacin, but only if administered early

Study II A: Experiment 1 Methods: Mouse models of anxiety-like behavior



- Open field test:
  Fewer entries into the center indicate anxiety-like behavior.

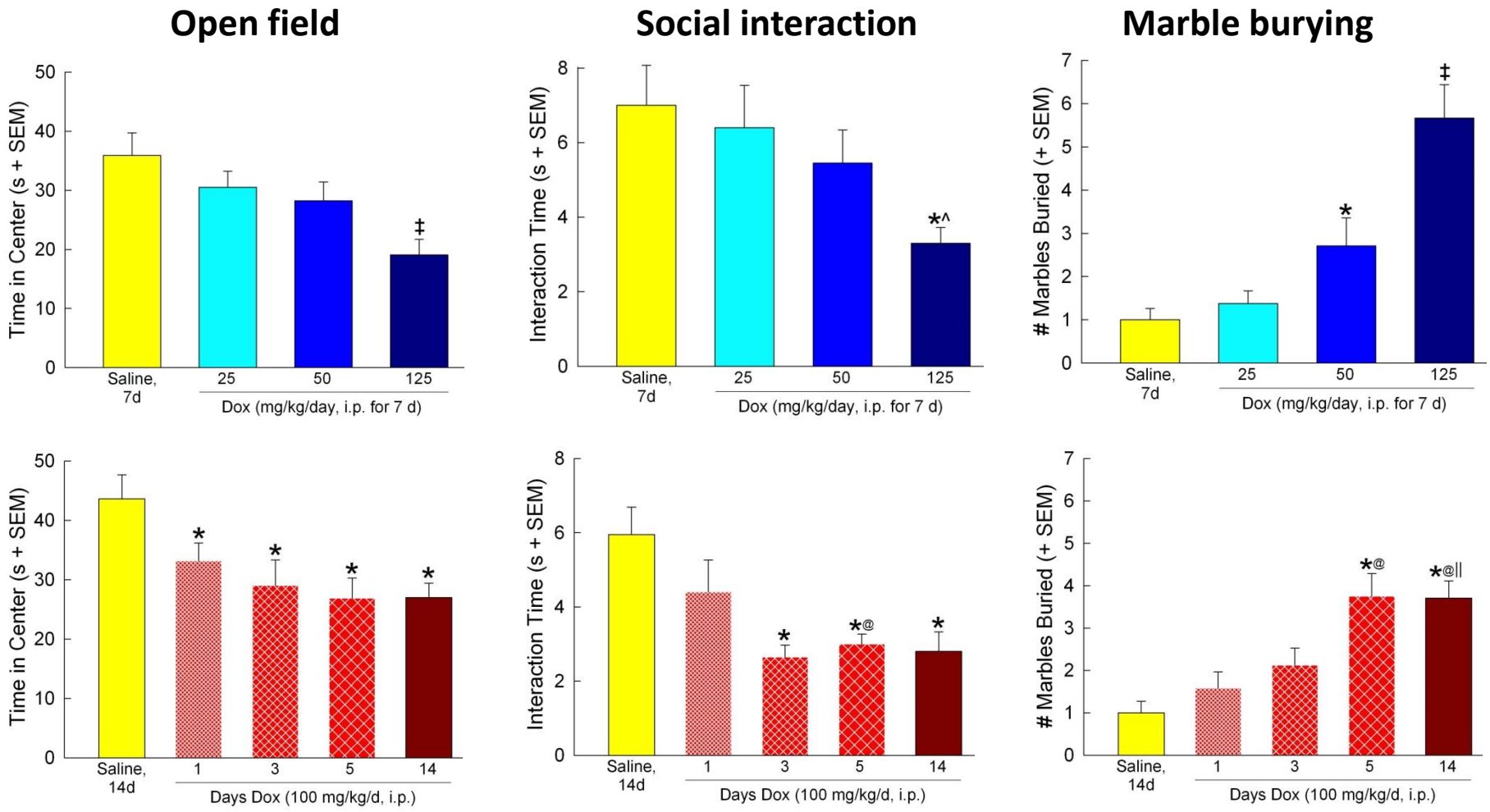
- Mouse social interaction test:
  Subject placed in corner of open field; weight matched novel mouse placed in the opposite corner, and interaction observed for 5 min.
  Less time spent in social behavior indicates anxiety-like behavior.



- Marble burying test:
  More marbles buried indicates anxiety-like behavior.

- Acoustic startle reflex (ASR):
  Longer time spent “frozen” indicates anxiety-like behavior.

Study II A: Increased exposure to Tat protein increases anxiety-like behavior in GT-tg mice

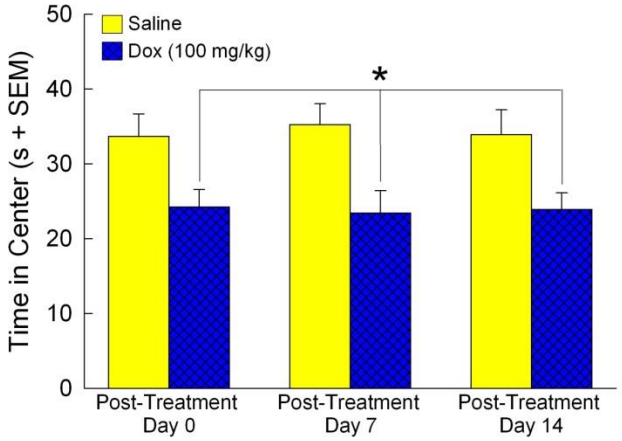


- C57BL/6J mice show no differences between saline and Dox treatment

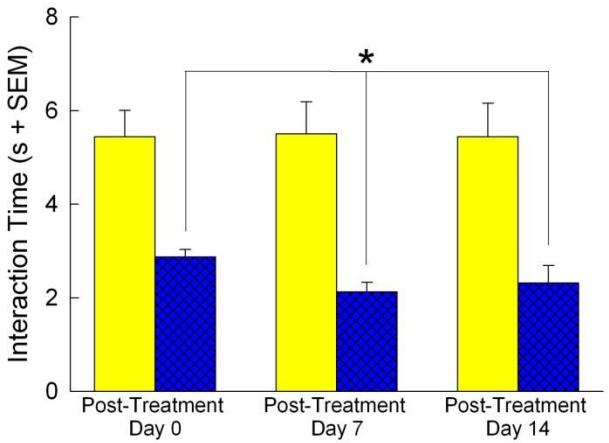
Paris et al, Psychopharmacology (Berl), ***231:*** *2014*

Study II A: Anxiety-like effects resulting from exposure to Tat protein endure at least two weeks after induction

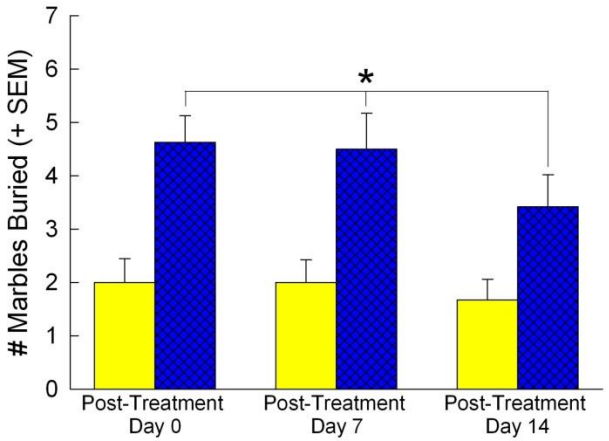
Open field



Social interaction



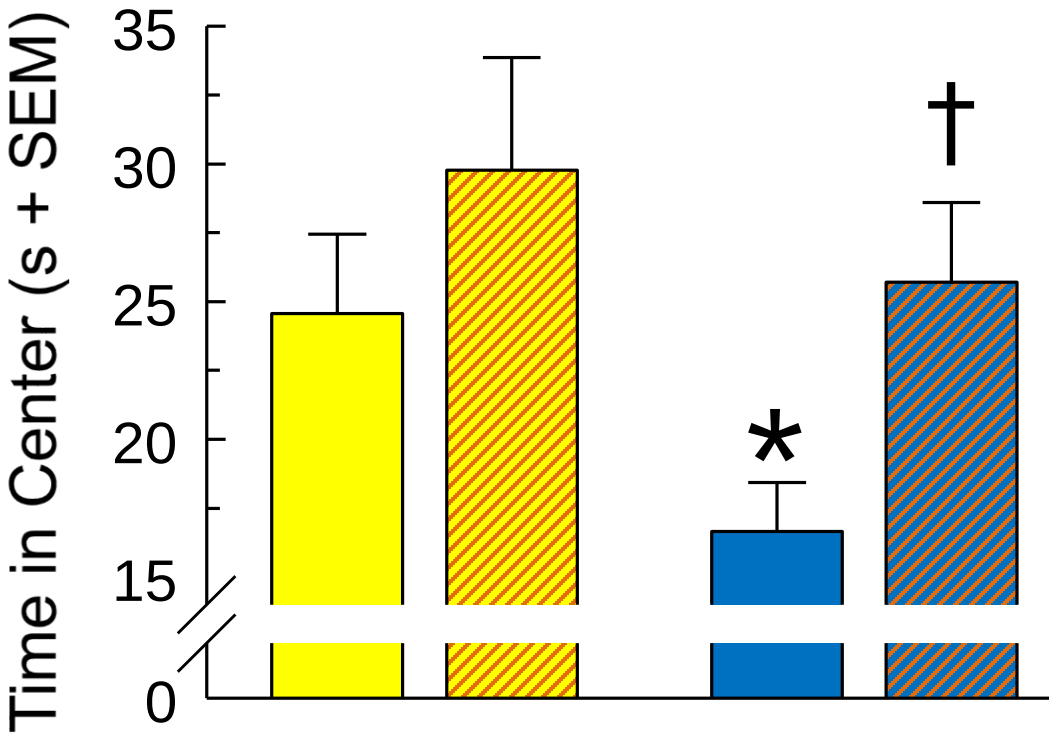
Marble burying



- Separate cohorts tested to be certain to avoid test decay
- C57BL/6J mice show no differences between saline and Dox treatment

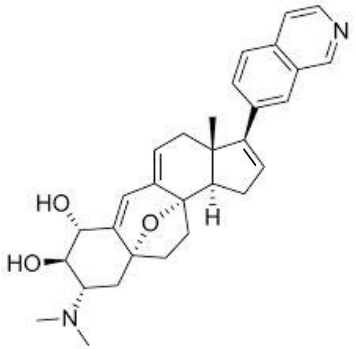
Paris et al, Psychopharmacology (Berl), ***231:*** *2014*

Study II A: Prevention of Tat-induced anxiety-like behavior in the open field assay following daily co-administration of didehydro-Cortistatin A (dCA)

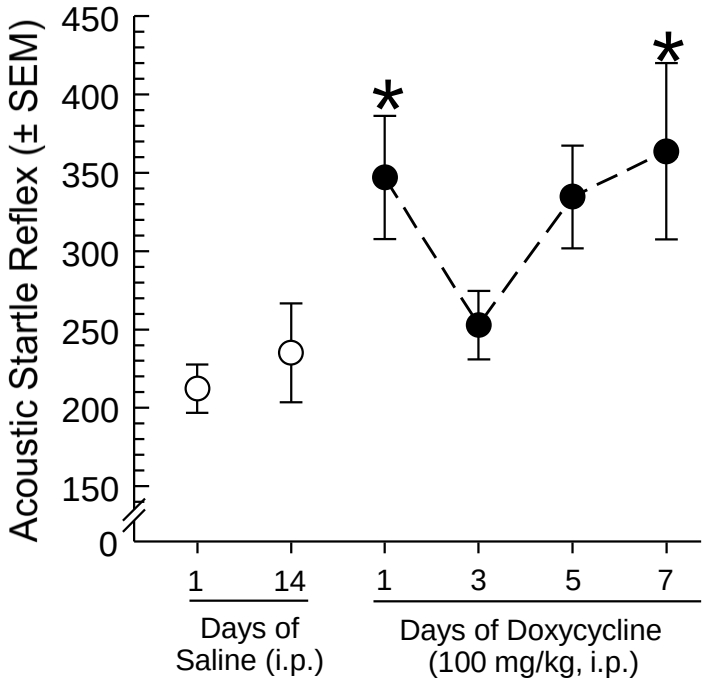


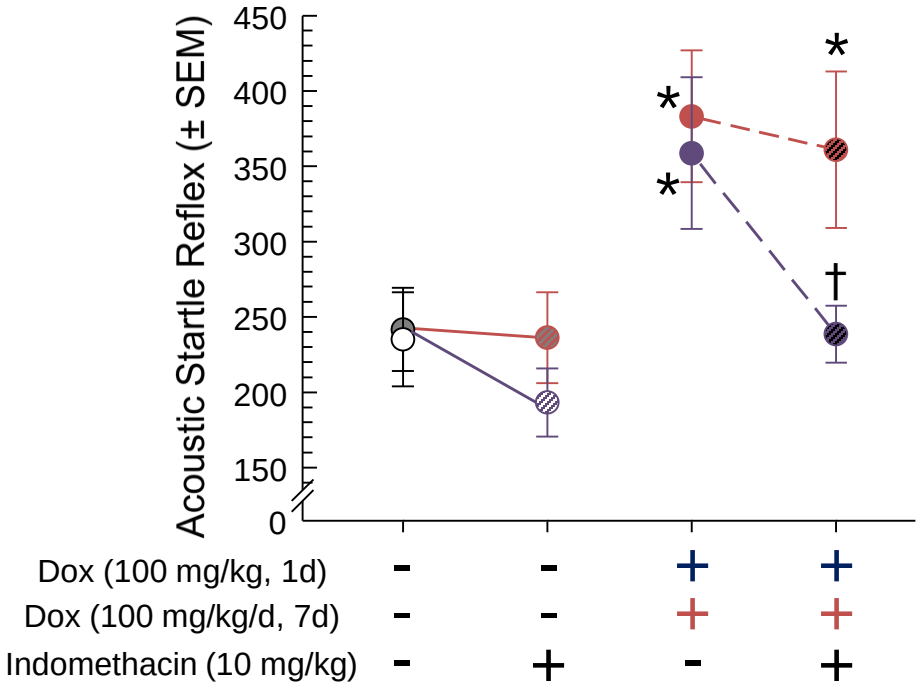
Daily treatment for 5 d with:

- Vehicle (i.c.v.) / Saline, i.p.
- dCA (10 nmol, i.c.v.) / Saline, i.p.
- Vehicle (i.c.v.) / Dox (100 mg/kg, i.p.)
- dCA (10 nmol, i.c.v.) / Dox (100 mg/kg, i.p.)



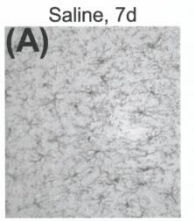
Study IIA: Exposure to Tat protein impairs the acoustic startle reflex in an exposure-dependent manner reserved by indomethacin treatment



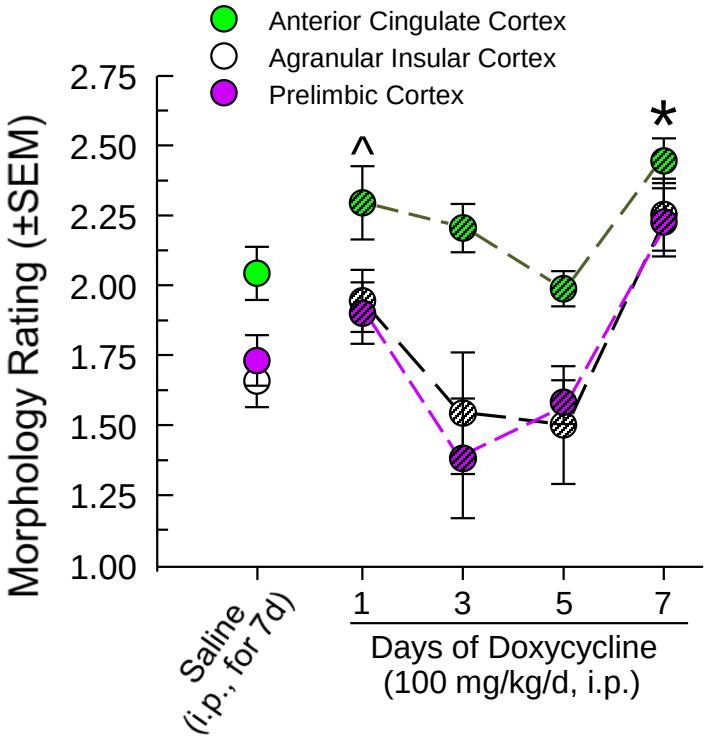


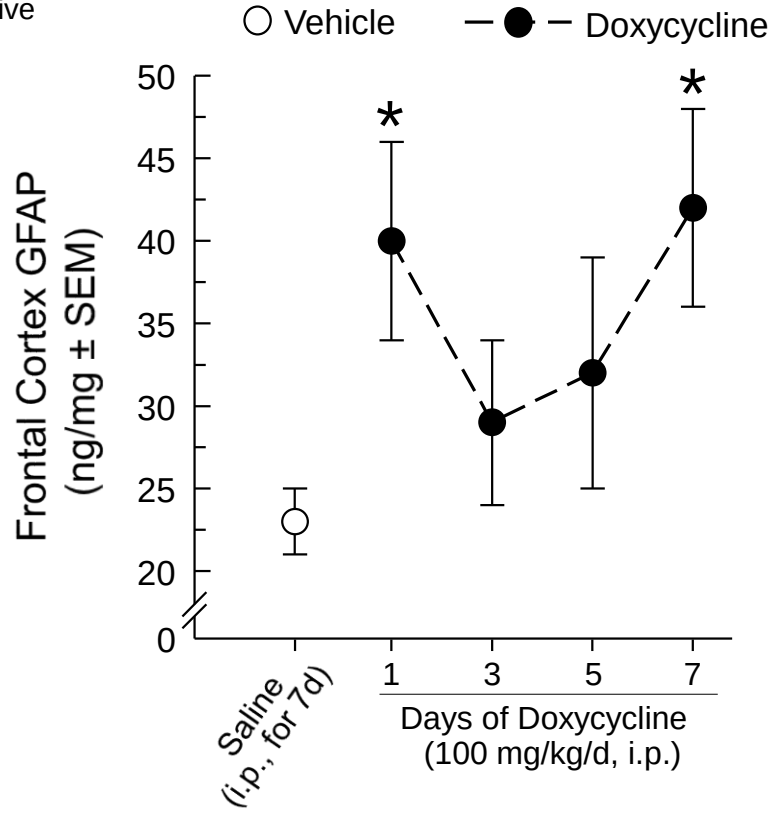
Paris et al, Behav Brain Res, ***291:*** *2015*

Study II: Exposure to Tat protein increases activation of Iba1-labeled microglia and GFAP content in frontal cortex of GT-tg bigenic mice in a time-dependent manner



Morphology rating
(Davis et al., 1994):
1 = resting
2 = early activated
3 = activated/reactive
4 = reactive



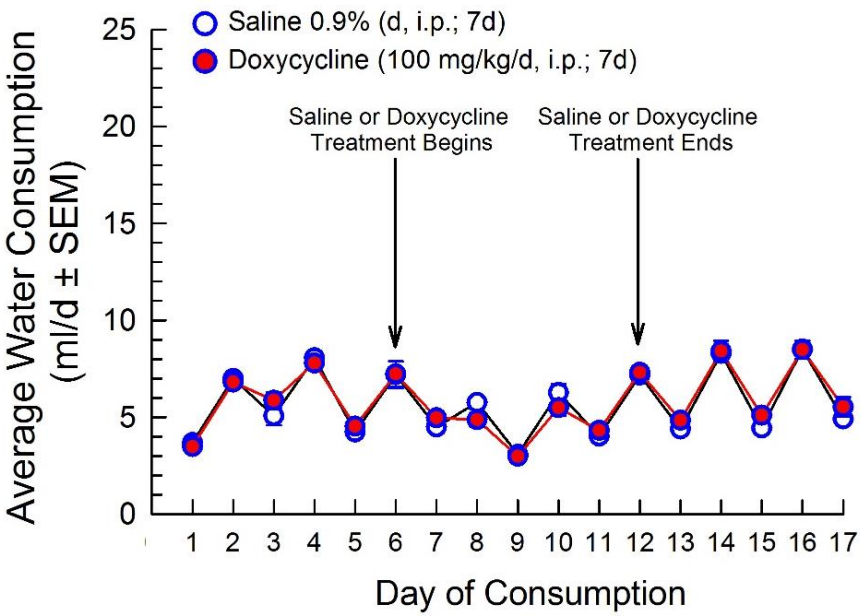


Paris et al, Behav Brain Res, ***291:*** *2015*

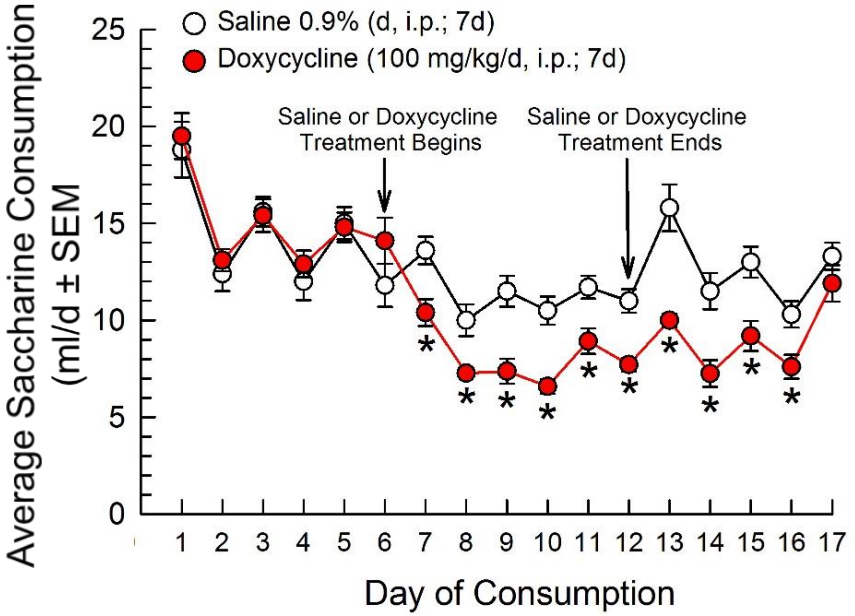
Study II B: Exposure to Tat protein decreases consumption of saccharine, but not water, in a two-bottle choice test

- Saccharine consumption test:
  Train individually-housed mice 4 days with two bottles of water,
  replace one with saccharine (0.2%) for testing
  Decreased saccharine consumption indicates anhedonia and depression-like behavior.

Bottle A: water

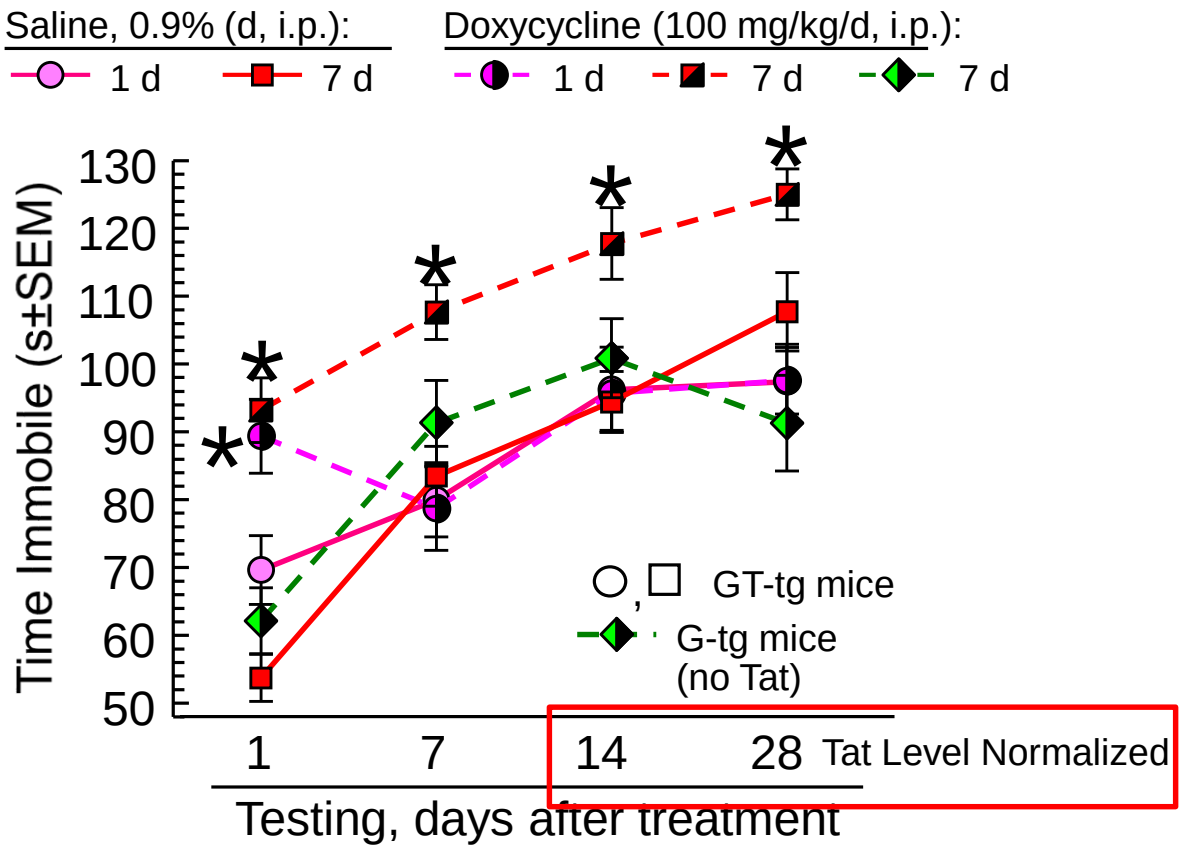


Bottle B: Saccharine (0.2%)

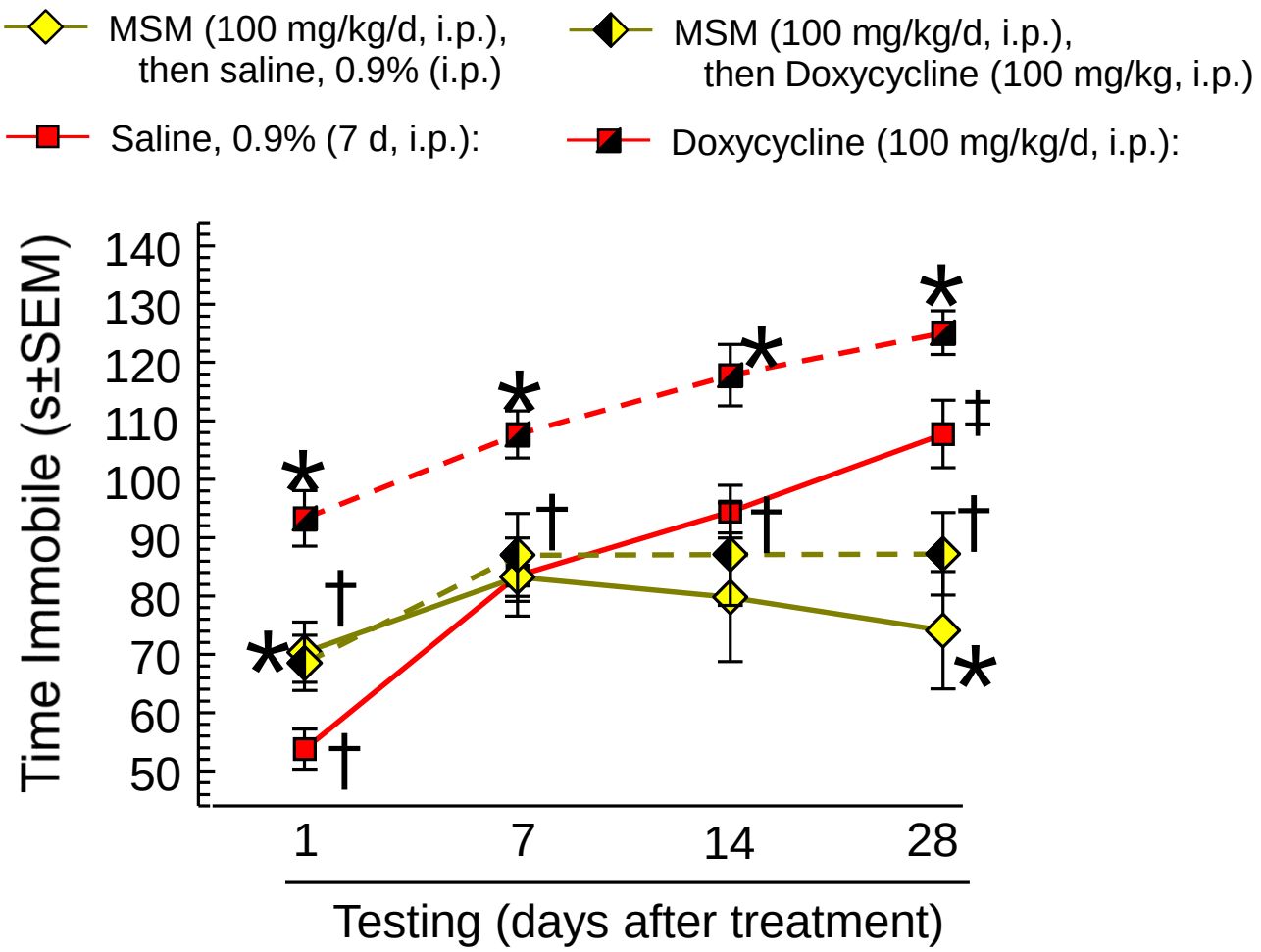


Study II B: Exposure to Tat protein increases depression-like behavior in the tail-suspension test in a persistent, exposure-dependent manner

- Tail-suspension test: suspend by tail for 5 min
  Increased time immobile indicates depression-like behavior.



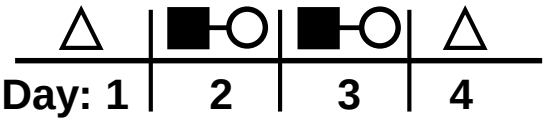
Study IIB: Co-treatment with methylsulfonylmethane (MSM) mitigates Tat-induced depression-like behavior in the tail-suspension test



Study II: Summary findings

- Mice expressing Tat protein demonstrate increased anxiety- and depression-like behavior in a dose- and duration-dependent manner
- Anxiety- and depression-like effects of Tat exposure persist for at least two weeks after completion of doxycycline treatment… and beyond point Tat protein is detected in brain
  - May signify progression from signaling to neurodegenerative effects?
- Duration of impaired startle reflex varies with duration of Tat exposure
  - Matches pattern of inflammation measured by Iba-1 labeled microglia and GFAP in brain
  - ASR deficits caused by brief exposure to Tat are reversed by indomethacin treatment, but this is ineffective after 7 days' Tat induction.
- Tat-induced immobility in the tail-suspension test was mitigated by co-treatment with methylsulfonylmethane

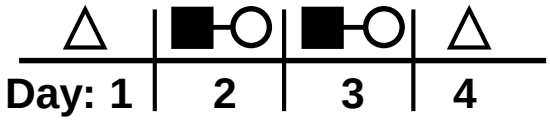
Study III Method: Conditioned place preference



△ = Place preference test (30 min)

■—○ = Cocaine place conditioning, then saline place conditioning (30 min each, 4 h apart)

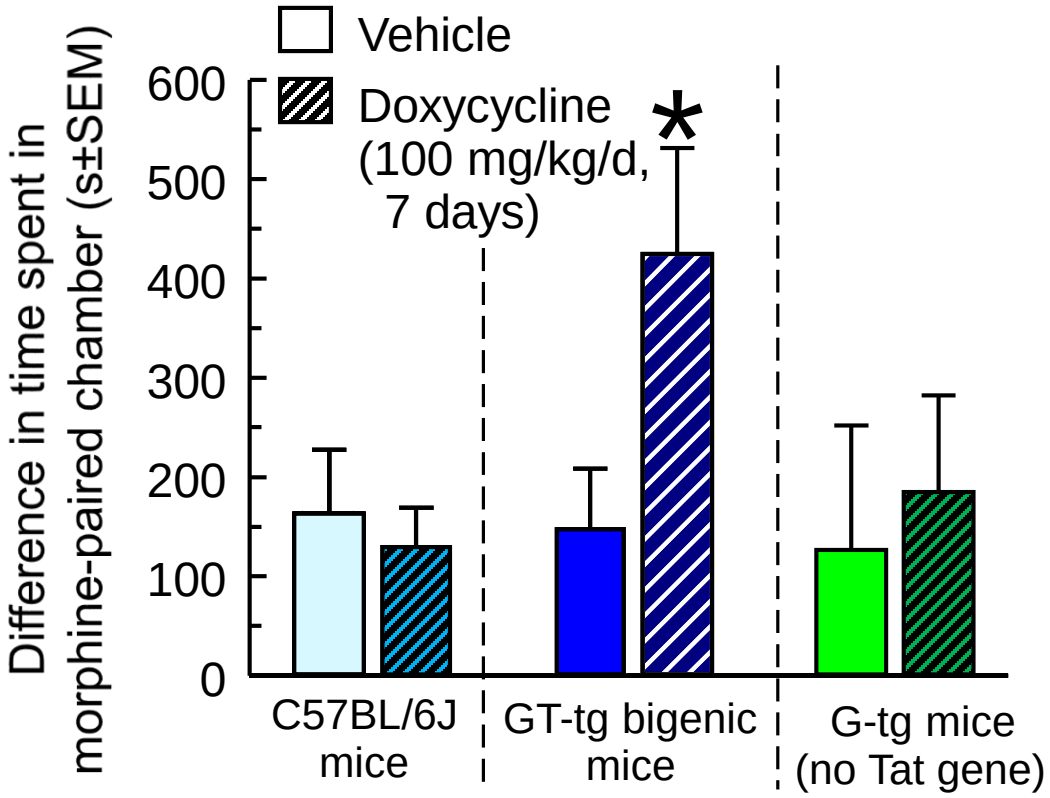
Study III: Tat expression potentiates the rewarding effects of morphine in the conditioned place preference (CPP) assay



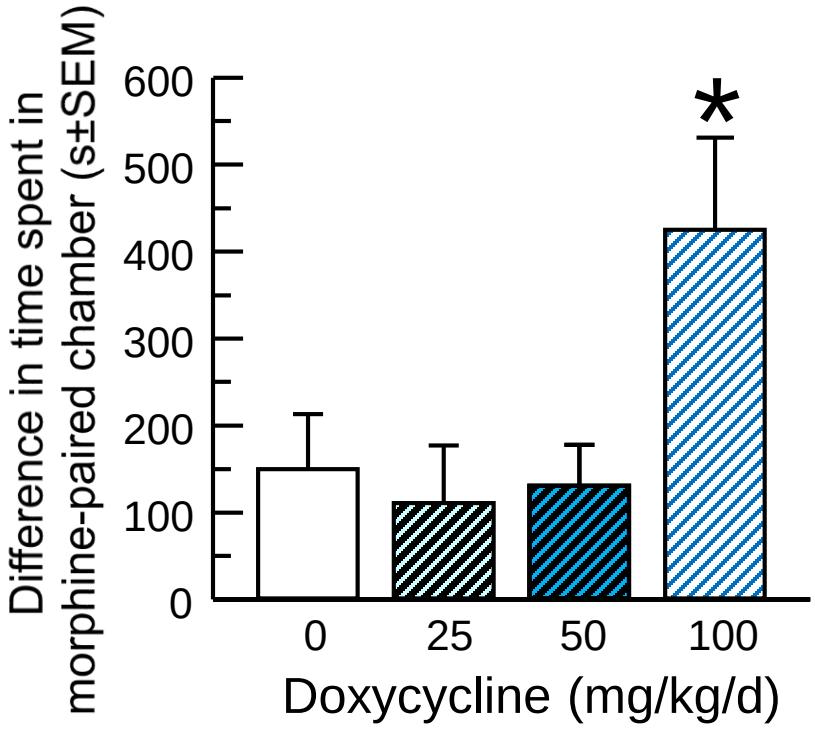
△ = Place preference test (30 min)

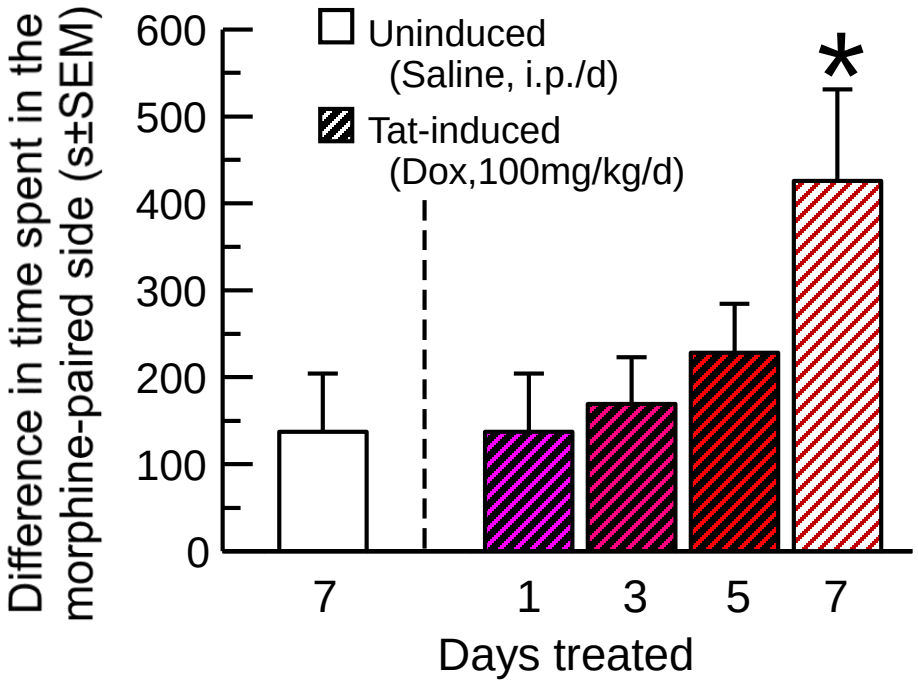
■—○ = Morphine place conditioning, then saline place conditioning (40 min each, 4 h apart)

(Mice pretreated 7 d with saline or Dox (@ 100 mg/kg/d)

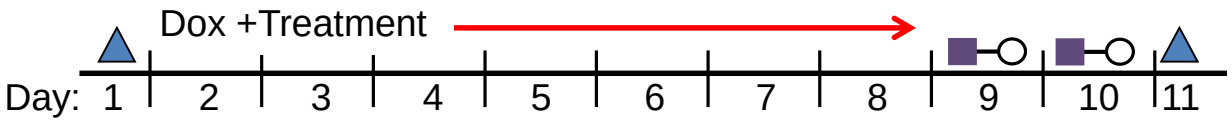


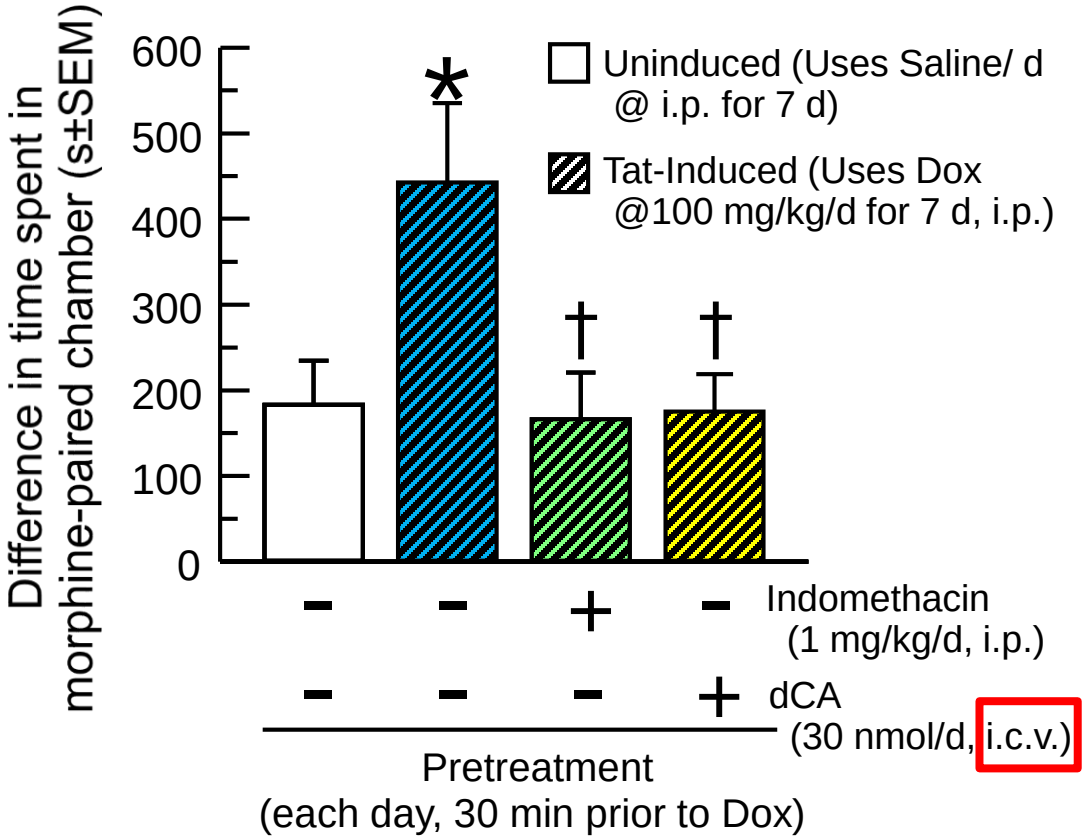
Study III: Tat-induced potentiation of morphine-CPP correlates with magnitude of Tat induction in GT-tg mice





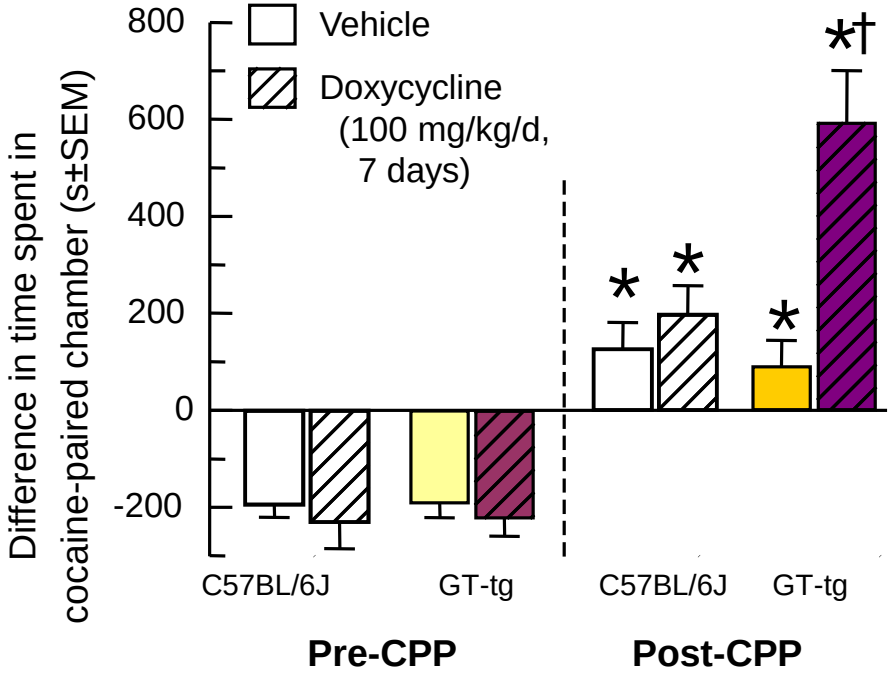
Study III: Daily pretreatment with indomethacin and didehydro-Cortistatin A (dCA) prevents Tat-induced potentiation of morphine-conditioned place preference

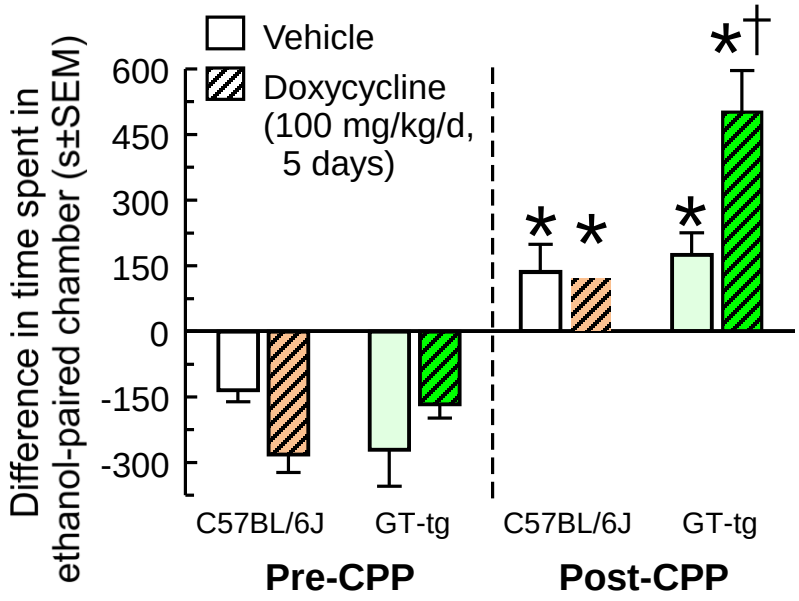




Vitaliano Nanoparticle delivery?

Study III: Exposure to Tat protein also potentiates cocaine- and ethanol-CPP in GT-tg bigenic mice

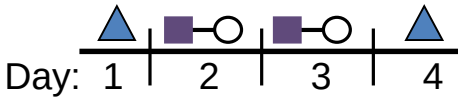


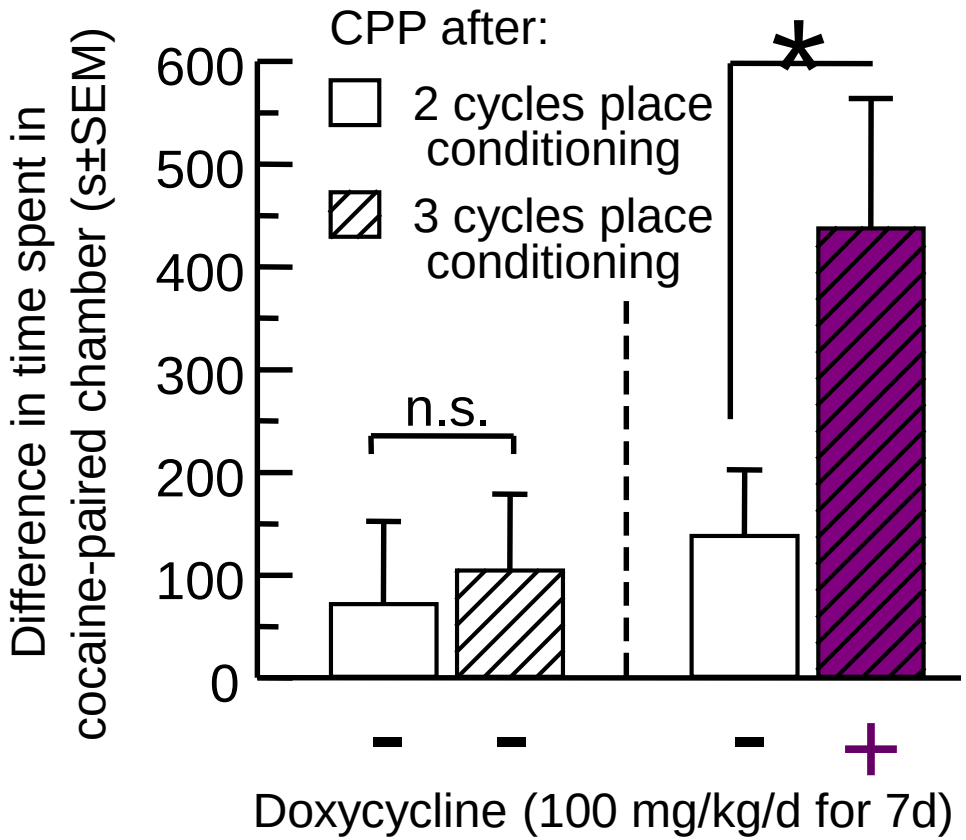


*Paris et al, Neuropsychopharmacology, **39:**2014*

*McLaughlin et al, Current HIV Research, **12:**2014*

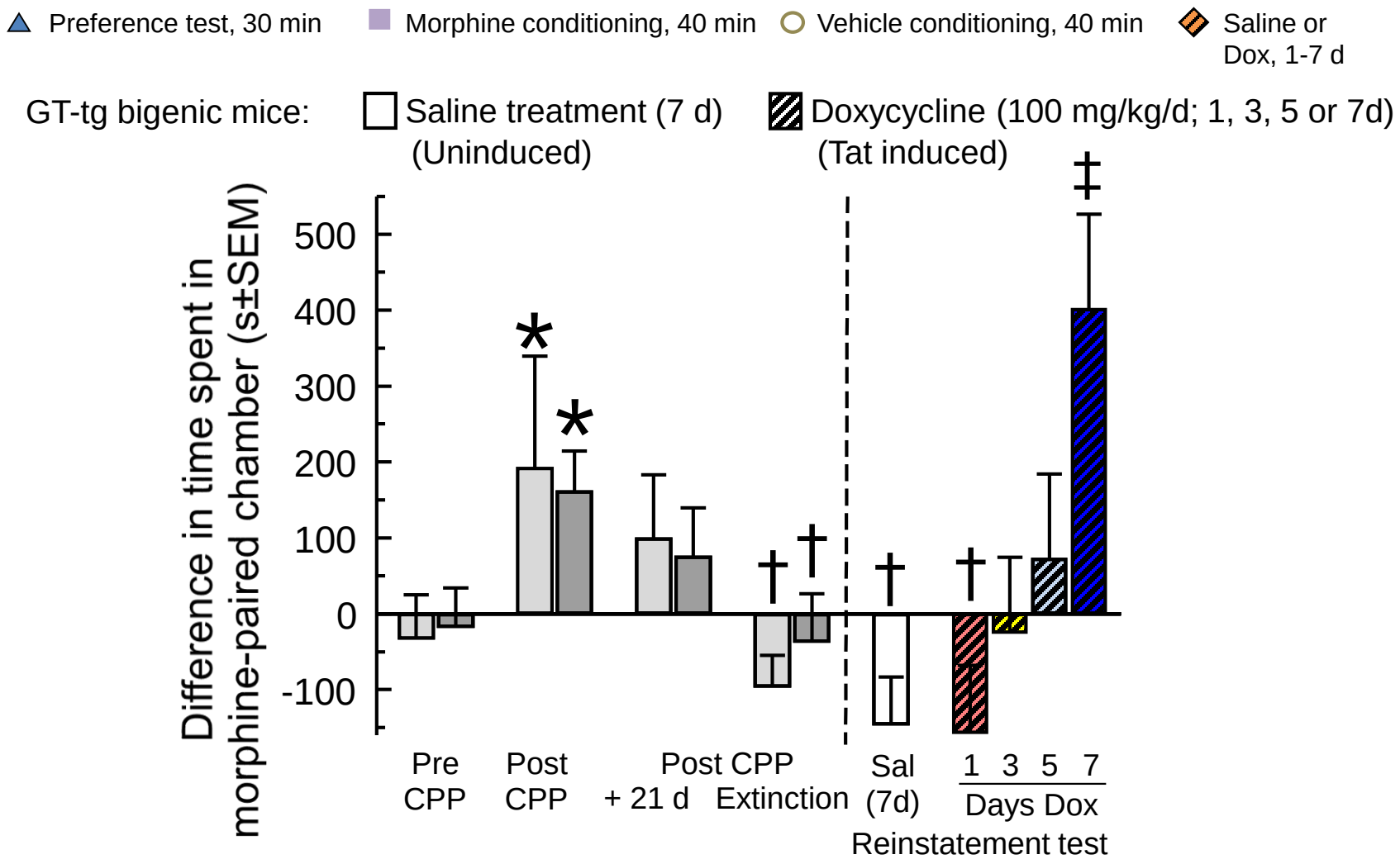
Study III: Acute induction of Tat subsequently potentiates established cocaine-CPP during re-exposure to cocaine



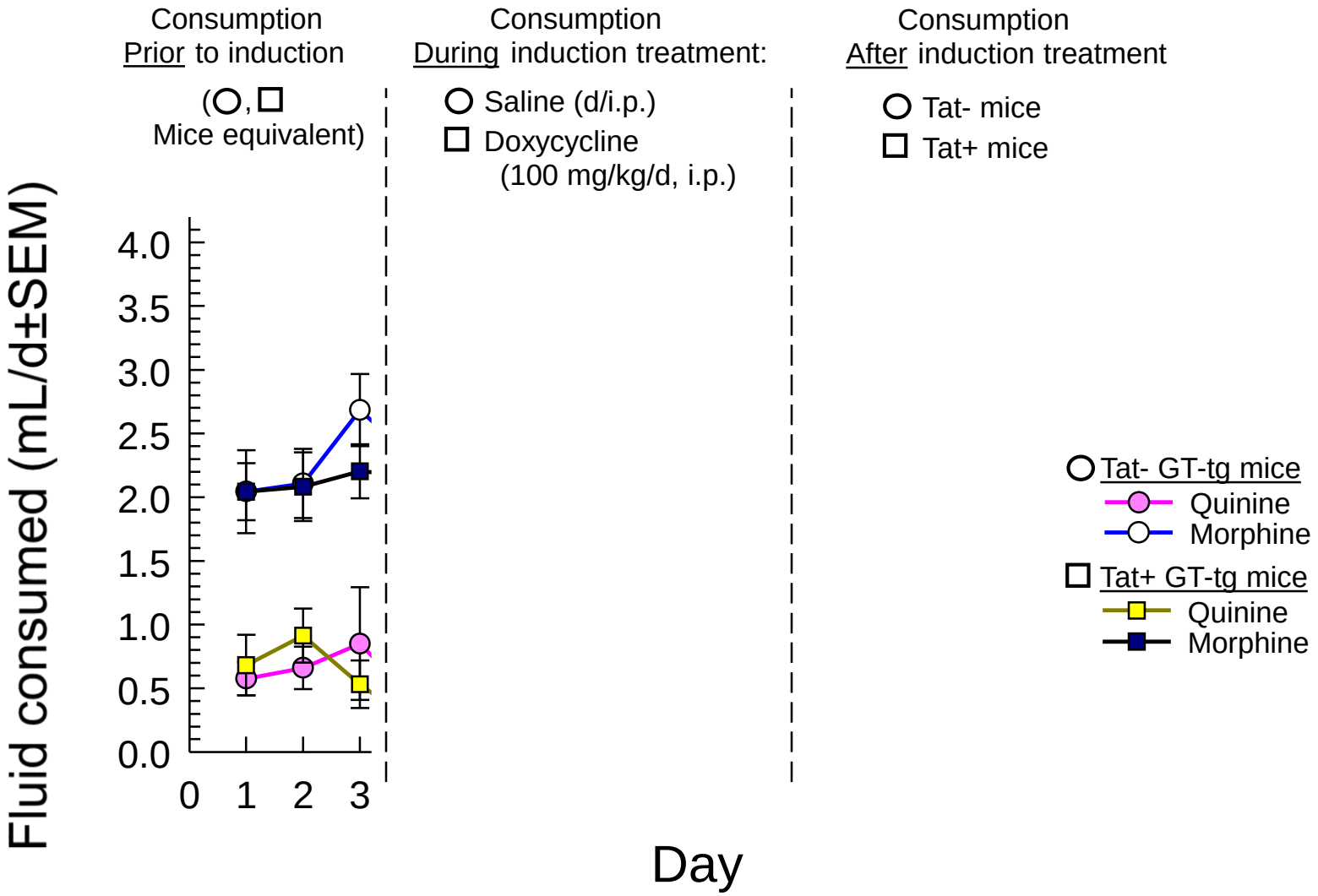


*Paris et al, Neuropsychopharmacology, **39:**2014*

Study III: Tat protein reinstates extinguished morphine-CPP in an exposure-dependent manner



Exp. IIIB: Exposure to Tat protein increases consumption of morphine in the two-bottle choice assay



Bottle A: Quinine (0.25 mg/ml) Bottle B: Morphine (0.4 mg/ml)

Conclusions

- Study 1: Effect of Tat expression on learning and memory and cognition:
  - Spatial learning (i.e., acquisition) was impaired in the Barnes maze
    - Reversal learning also impaired
  - Novel object recognition performance was impaired in Tat-induced mice for up to one month in an exposure-dependent manner
    - Pretreatment with Tat-inhibitor dCA prevented impairment of NOR
  - Pre-pulse inhibition was impaired in Tat-induced mice

- Study 2: Effects of Tat expression on disorders of mood:
  - Tat expression produces anxiety-like behavior in exposure-dependent manner
  - Low or high exposure to Tat amplified the acoustic startle reflex
    - Low or high Tat exposure activated microglia and increased GFAP
    - Indomethacin mitigated effects of brief, but not prolonged, Tat exposure
  - Tat exposure increased the time spent immobile in the mouse tail-suspension test
    - Persistence of depression-like effects dependent on duration of Tat exposure
    - Methylsulfonylmethane mitigated depression-like effects of Tat exposure

Conclusions

- Study 3: Effects of Tat expression on morphine reward:
  - Exposure to Tat protein potentiates the locomotor effects of morphine
    - Effect consistent with earlier cocaine results (Paris et al., 2014)
  - Effects of Tat expression on morphine reward:
    - Tat expression potentiates morphine CPP in exposure-dependent manner
    - Effect not seen in saline treated GT-tg mice, or Dox-treated G-tg or C57BL/6J mice
    - Increases place conditioning effect of additional (normally inconsequential) exposure to cocaine
    - Causes reinstatement of extinguished morphine-seeking behavior
    - Increases voluntary consumption of morphine…
- Together, these results suggest that exposure to Tat protein is sufficient to:
  - Promote disorders of mood associated with NeuroAIDS and HAD
  - Play a role in the development of cognitive impairment
  - Potentiate the rewarding effect of abused substances, and produce relapse to drug-seeking behavior in abstinent subjects.

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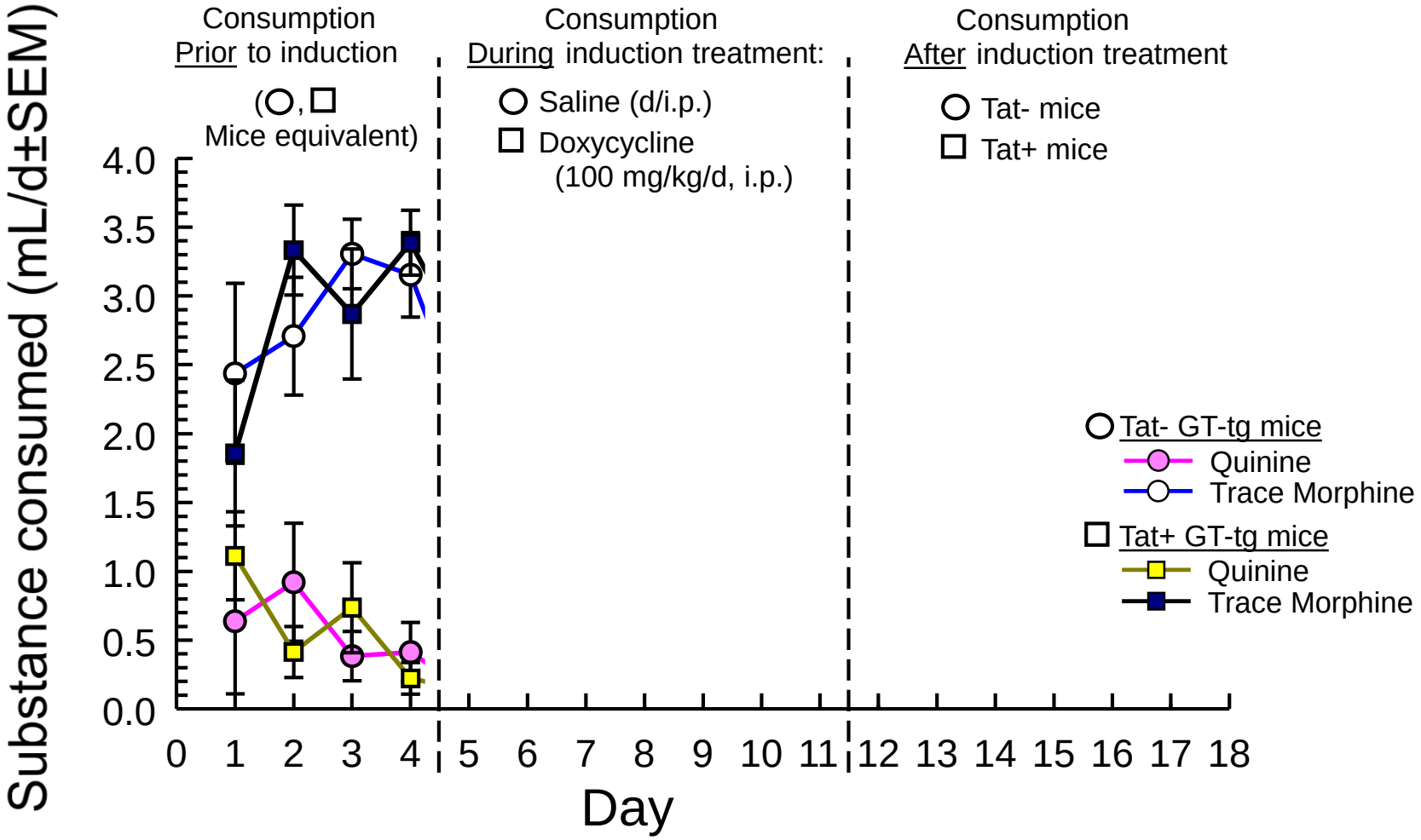
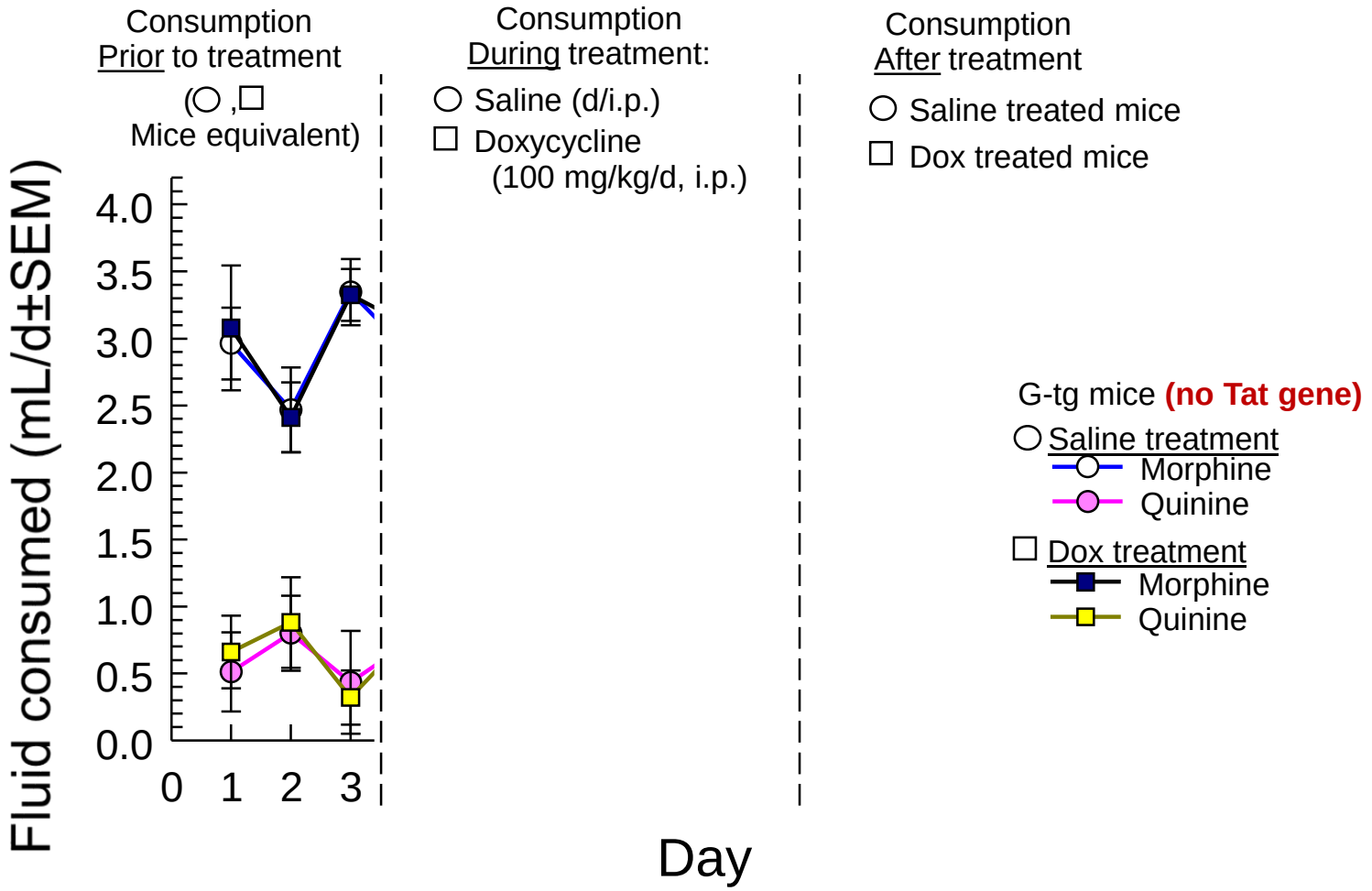
Experiment III: Exposure to Tat protein reduces consumption of water in the two-bottle choice assay



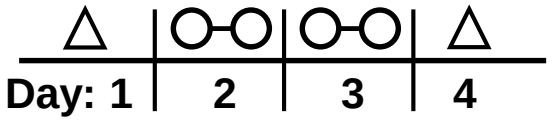
Bottle A: Quinine (0.25 mg/ml) Bottle B: Trace Morphine (0.13 µg/ml)

Exp. IIIB: In mice lacking Tat, exposure to Doxycycline briefly reduces consumption of morphine in the two-bottle choice assay



Bottle A: Quinine (0.25 mg/ml) Bottle B: Morphine (0.4 mg/ml)

Study III: Doxycycline-induced (100 mg/kg/d, i.p.; 7 d) expression of Tat protein has no effect on saline conditioned place preference



△ = Place preference test (30 min)

○–○ = Saline place conditioning, then saline place conditioning (30 min each, 4 h apart)