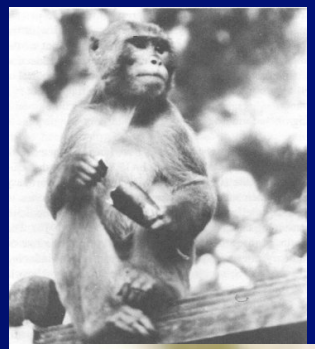
Using Neuroimaging to Explore Addiction in Animal Models



NHPs in neuroimaging can be used for……

- Radiotracer development / Occupancy studies

- Modeling human drug addiction and other brain diseases

Radiotracer development

Purpose:

- **Test new ligands**
- **Characterize and develop the ligands**
- **Data in NHP required for FDA application to take to humans**

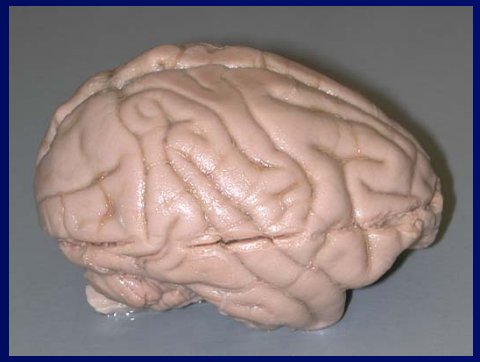

AND THEN THEY GAVE ME THIS NEW DRUG AND I THOUGHT WHAT THE HECK, WHY NOT, I'VE TAKEN EVERYTHING ELSE ...
ShieLL
LAB RAT REHAB

Brain comparisons

Human

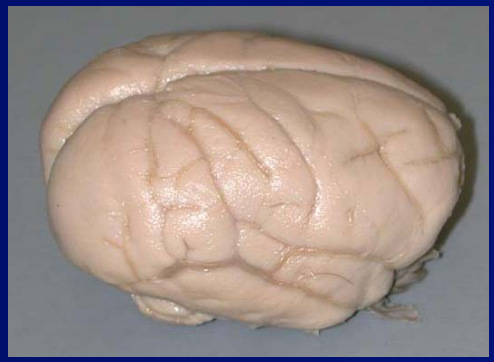
Length - 15 cm
Weight - 1400 g

Baboon

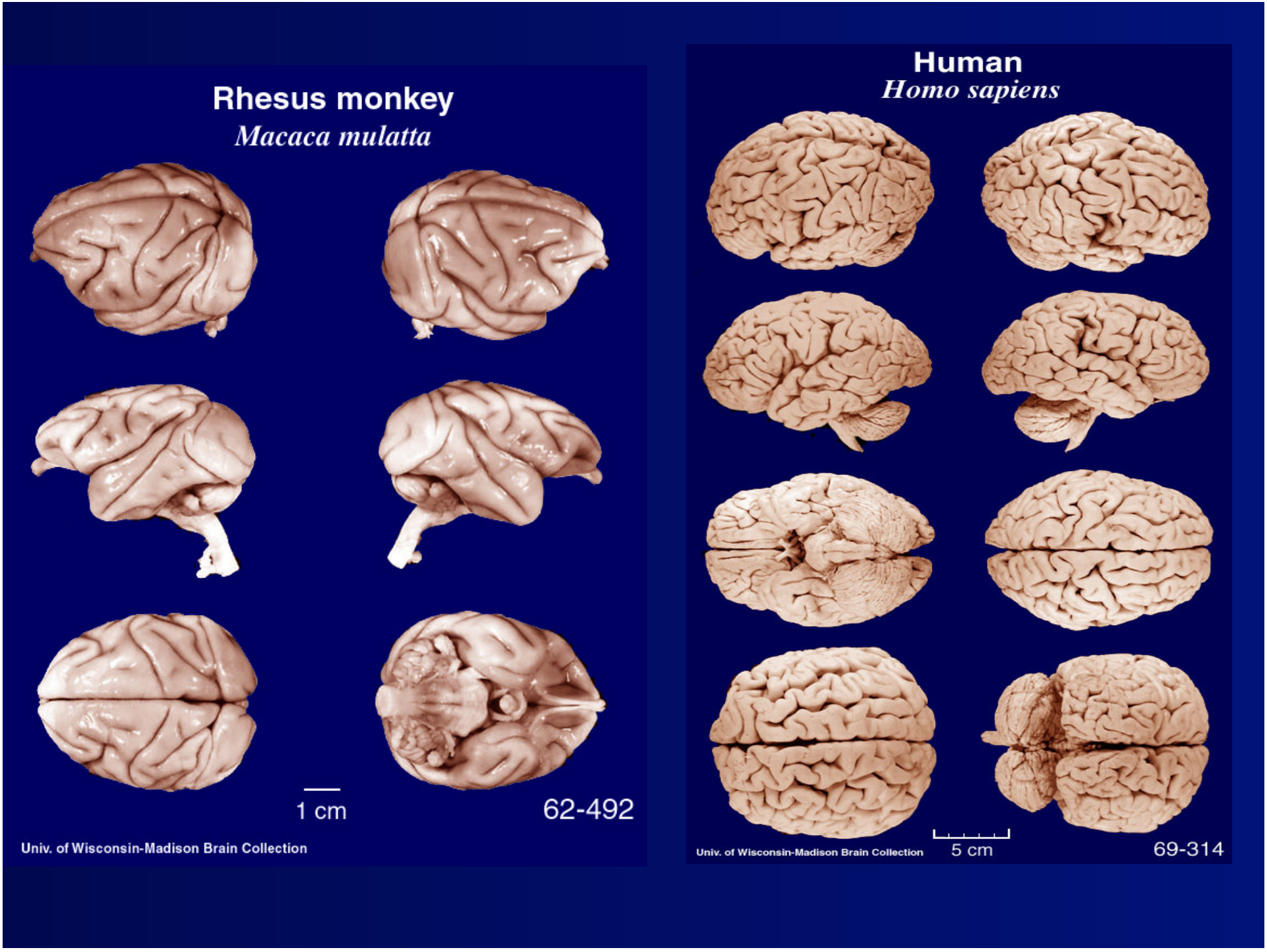


Length - 8 cm
Weight - 140 g

Monkey



Length - 5 cm
Weight - 100 g


Rhesus monkey
Macaca mulatta
1 cm
62-492
Univ. of Wisconsin-Madison Brain Collection
Human
Homo sapiens
5 cm
Univ. of Wisconsin-Madison Brain Collection
69-314

Rat Brain
2 g

Imaging animals

- SPECT
- PET
- MRI
- fMRI
- Small animal, e.g., microPET, microSPECT
- Bonus: microdialysis/voltammetry can be done at the same time

Why would you do autoradiography?

If you think your radiotracer is influenced by endogenous compound, you can wash it out, see how it compares to imaging study.

Disadvantages of imaging animals

- Need to anesthetize animals so they do not move! This may alter brain function.
- ~~Very~~ limited success at imaging conscious animals
- Can be more costly than imaging humans

Advantages of NHP models of addiction

- Self-administration
- Complex social behaviors/individual differences (hierarchy)
- Image adolescents
- Image drug-naïve animals and then while taking drug (pre-existing vs. consequence question)
- Can learn complex behaviors to obtain drugs
- Experimental control, e.g., drug history
- Longitudinal studies can be performed with neuroimaging but not autoradiography

Animal models of drug abuse-face validity?

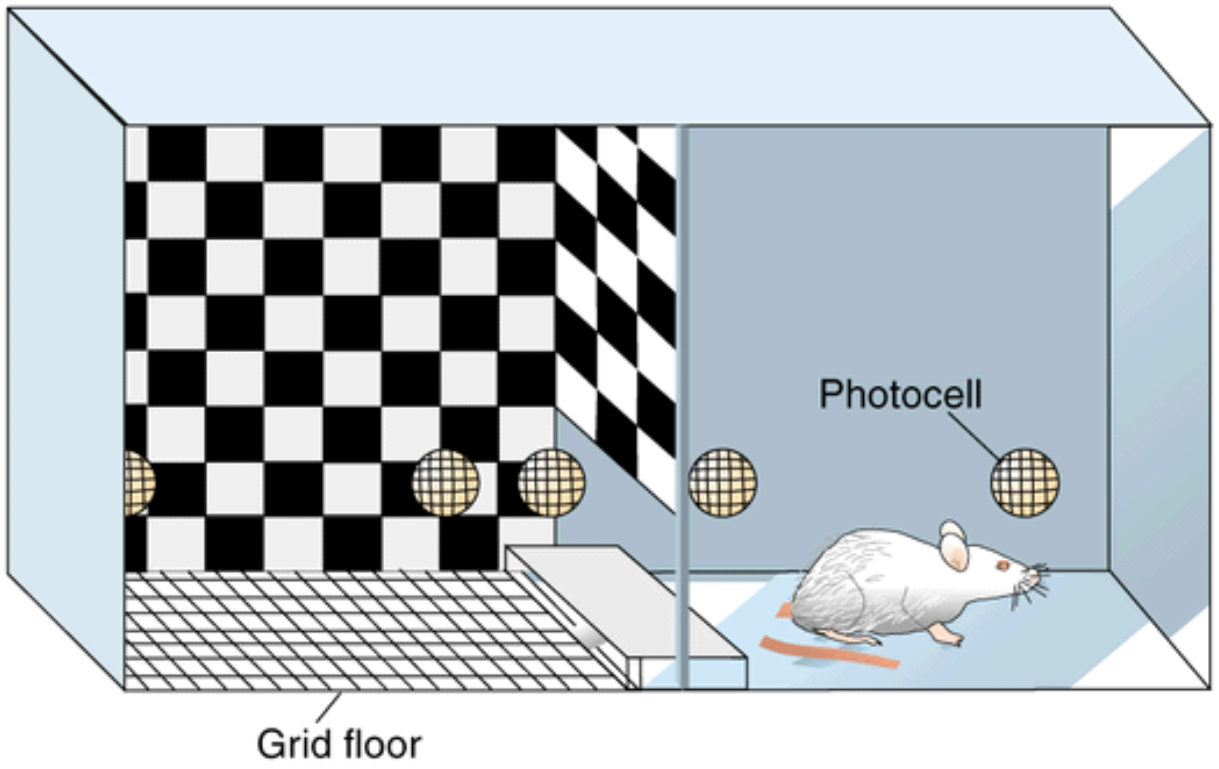
- Valid predictors of human drug abuse
- Animals self-administer the same drugs by the same routes of administration
- Also used to examine environmental variables and vulnerability to drug abuse
- Individual differences are clear, e.g., response to stress

Paradigms used to model addiction

- Self-Administration – gold standard
  - Oral
  - IV
  - Inhalation, e.g., smoked cocaine
- Conditioned Place Preference (CPP)
- Investigator administered drugs, e.g., ethanol and nicotine vapor chambers

Conditioned Place Preference

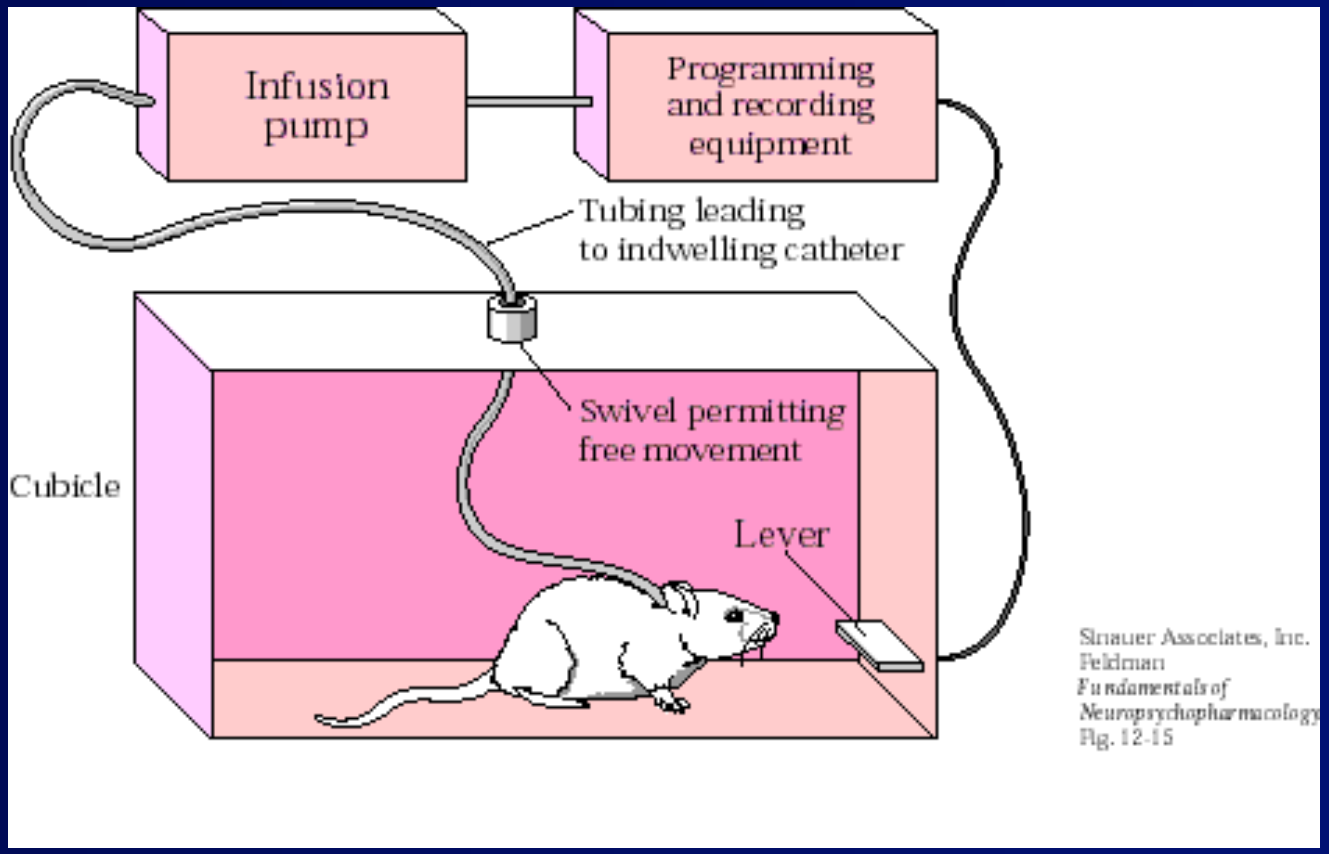
► The Conditioned Place Preference Procedure



Source: Adapted from Feldman, R.S., Meyer, J.S., and Quenzer, L.F. *Principles of Neuropsychopharmacology*. Sunderland, MA: Sinauer Associates, 1997.

Oral self-administration

Intravenous self-administration



Rodent self-administration chamber

Variations that increase validity

- Inclusion of nondrug alternative reinforcers, e.g., preferred food, running wheel, friends

- Choice between drug and food
  - Low drug dose → monkey chooses food
  - Higher dose → monkey chooses drug

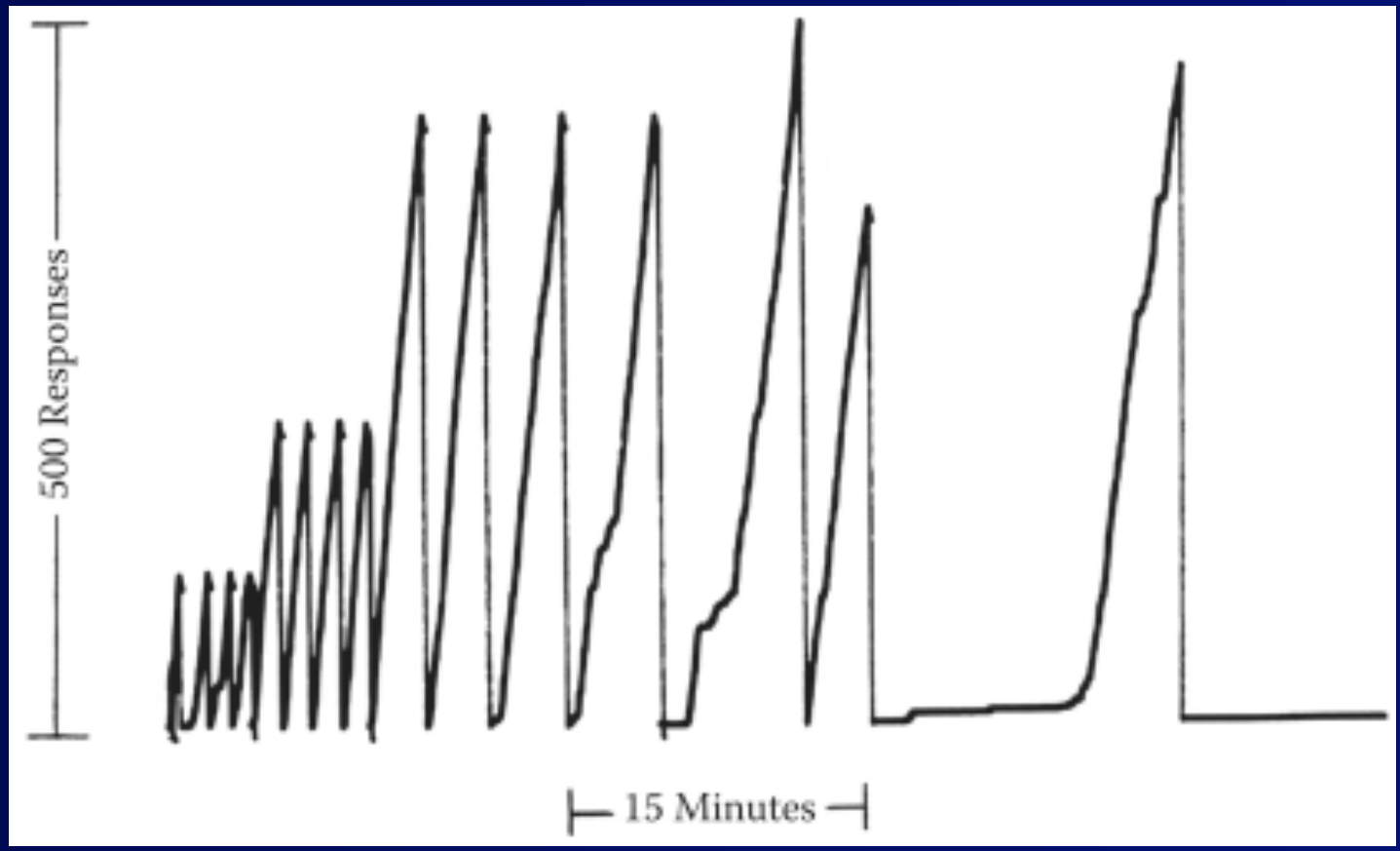
- Magnitude of the reinforcer, e.g., amount of food, amount of work or "cost" of the drug

Schedules of reinforcement

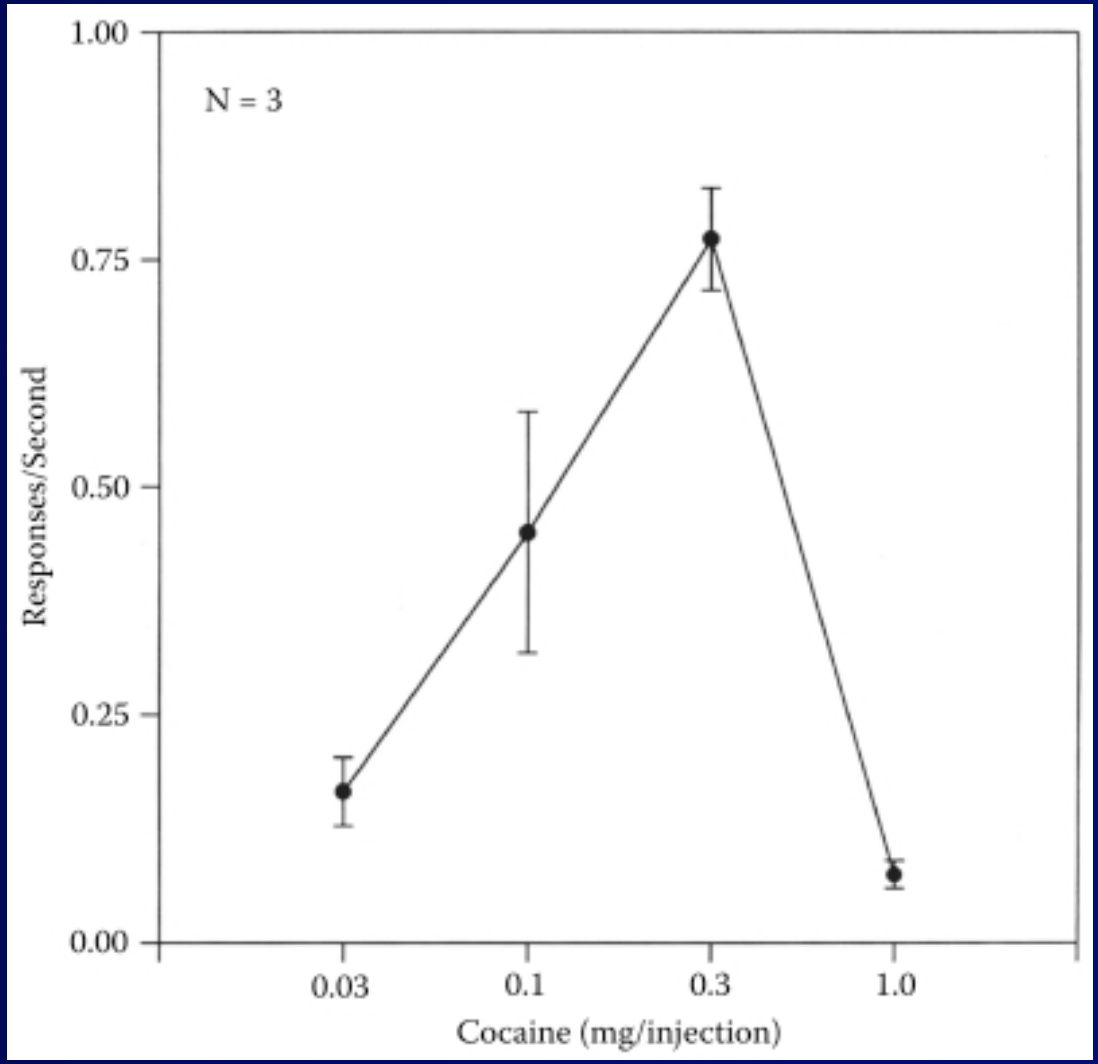
- **FR – fixed ratio, e.g., FR1, FR2, FR30**

- **PR – progressive ratio, e.g., a within session change in response requirement, a specified increment,** e.g., 10, or a doubling -2 4 8 16 32 64 128 256 512 1024 2048 4096 8192 16384
  - **Allows you to measure a "breaking point", a point of maximal responding**
  - **Is a measure of motivation**

PR Responding

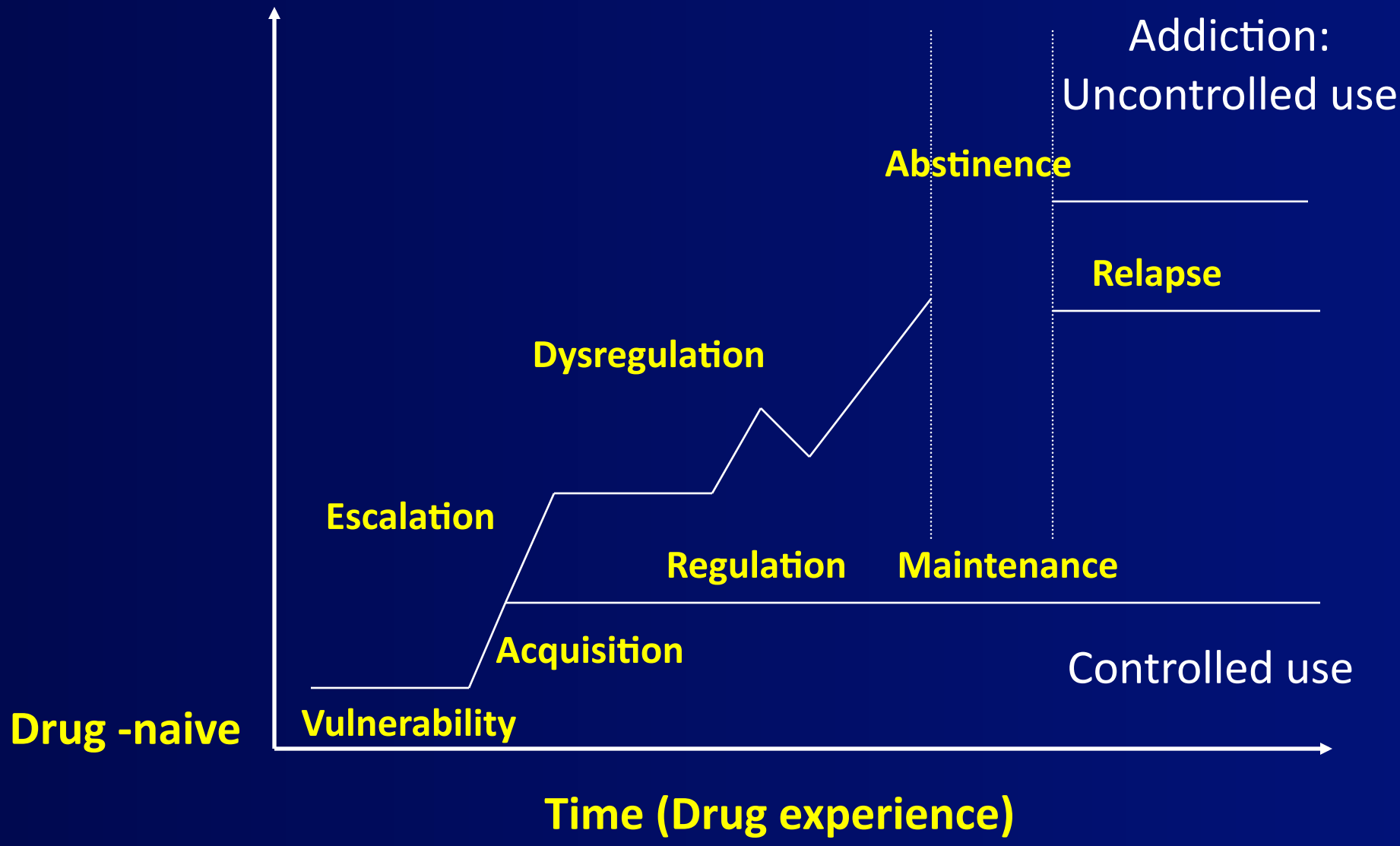


Inverted U



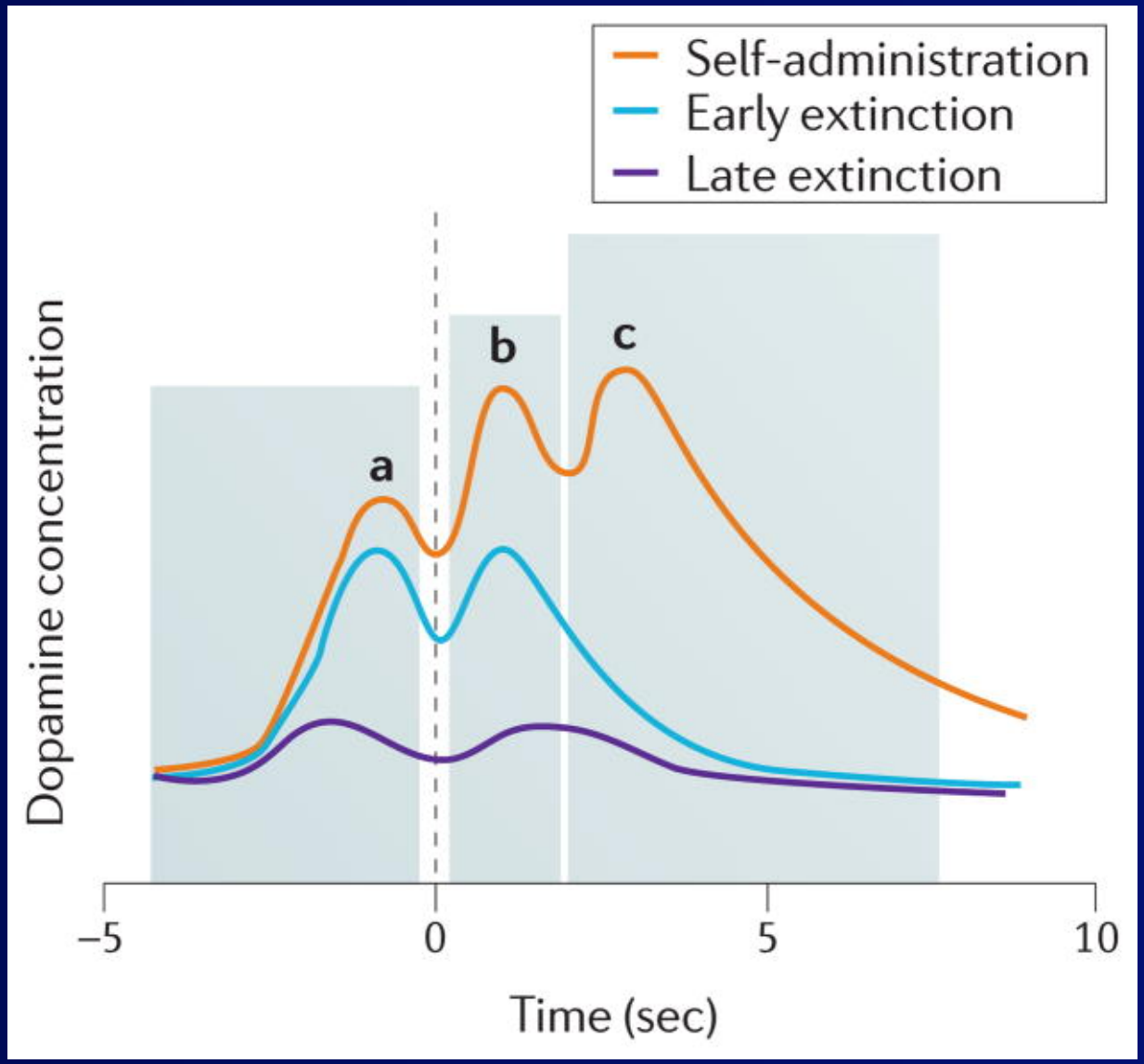
Howell and Fantegrossi,

Phases of drug addiction



How does Cocaine work?

- Cocaine binds to the dopamine, serotonin, and norepinephrine transporters
- Majority of research focuses on dopamine system
- Cocaine works by binding to the DAT and blocking the reuptake of DA from the synapse



Wise and **Kiyatkin**, Nat Rev, Neuro, 2011

What was the design of the Morgan….Nader study?

^{18}F Fluoroclebopride

A PET tracer used to measure D2 receptors

Outcome measure is the distribution volume ratio

$$\frac{\text{DV in region of interest}}{\text{DV in region devoid of receptors}}$$

DVR vs BP

DVR vs BP

\>>

DVR is DV(target) / DV(ref)

DV in a region with specific binding (and no nonspecific) is

$$DV(Target) = \frac{K1*(1 + k3/k4)}{k2}$$

whereas

DV in a region with no binding (k3=0) is simply

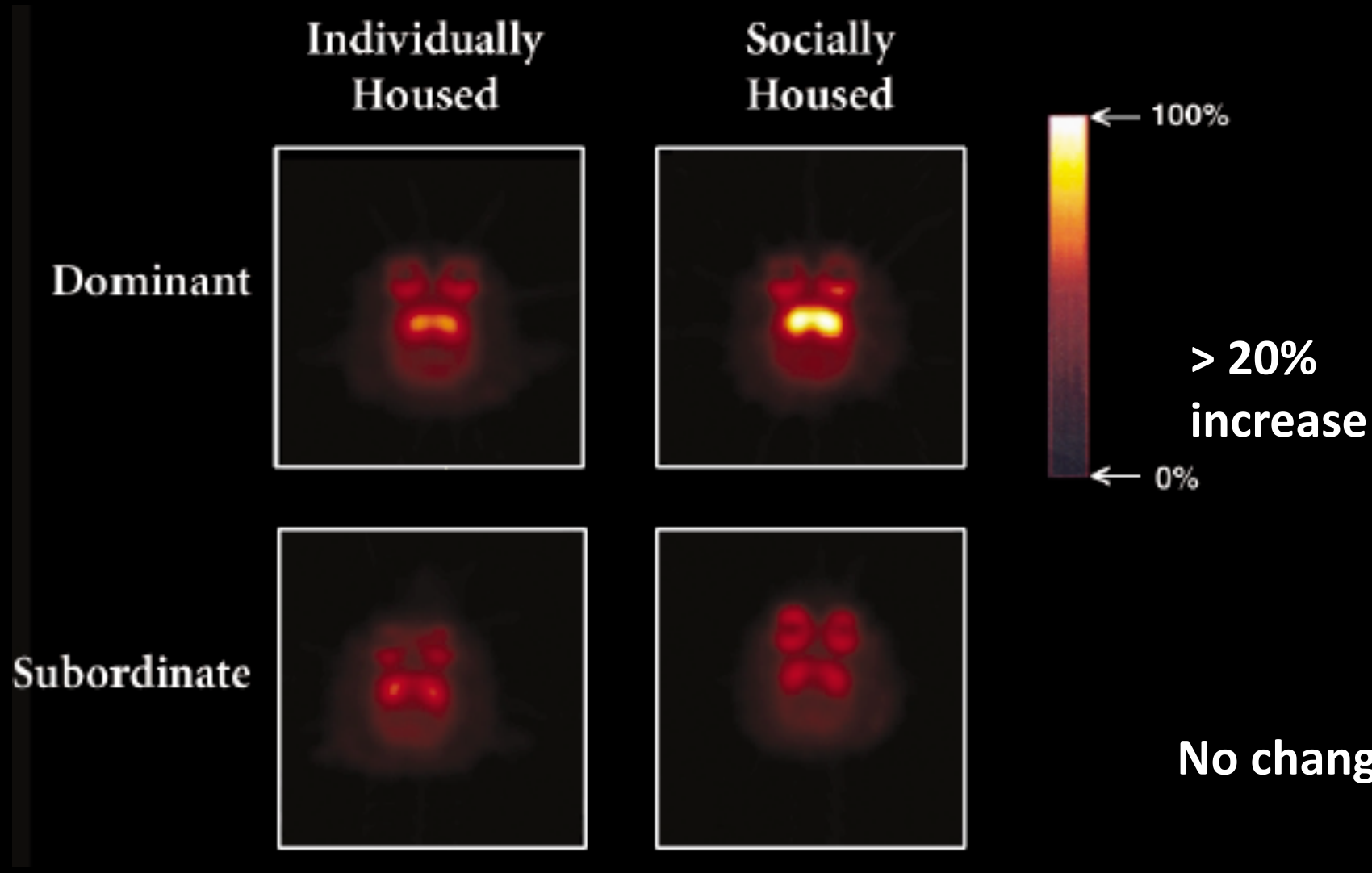
$DV(ref) = K1/k2$

So DVR = DV(target)/DV(ref) = (1 + k3/k4)

SO !!   DVR-1 = BP

BUT remember, this assumes no Nonspecific.

Social hierarchies influence D2 receptor availability



Morgan, Nat Neuro, 2002

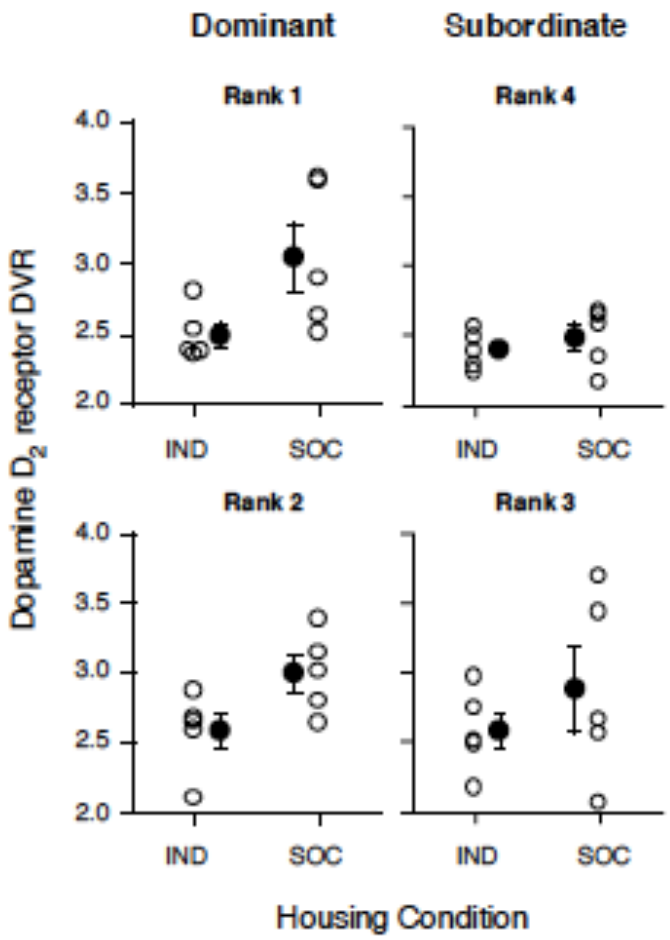


Fig. 2. [^{18}F]FCP binding potential changes as a function of social rank. Panels show the mean and individual [^{18}F]FCP DVR values for monkeys with different social ranks, while they were individually (IND) and socially (SOC) housed.

Subordinate monkeys self-administered significantly more cocaine than the dominant monkeys

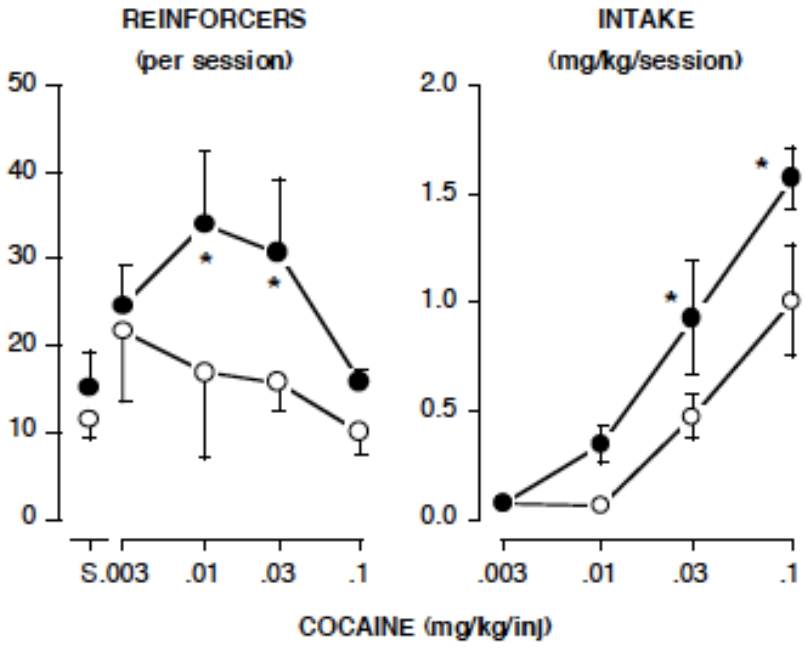


Fig. 4. Reinforcing effects of cocaine are greater in subordinate monkeys compared to dominant animals. Left, mean number of intravenous injections (either saline or various doses of cocaine) per session for 5 dominant (rank 1 and 2, white symbols) and 4 subordinate (rank 3 and 4, black symbols) monkeys. Right, mean intake per session for dominant (white symbols) and subordinate (black symbols) monkeys. Each dose was available for at least 7 sessions and until responding was stable. Data represent the mean of the last 3 days of availability for each animal. Asterisk indicates a statistically significant difference ($p < 0.05$) from dominant monkeys at that particular dose, and from the appropriate saline point.

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of Laboratory Animals as adopted and promulgated by the NIH, and with the approval of the Institutional Animal Care and Use Committee.

Behavioral profiles of socially housed monkeys. Monkeys were individually housed for approximately 10 months before the initial PET scans. The monkeys were placed into social groups of four after initial PET scans were completed. Approximately 20 behavioral observation

Increase in D2 receptors or decrease in DA?

- Group housing is analogous to enrichment for dominant monkeys…….
  - Treats
  - Grooming
  - Active

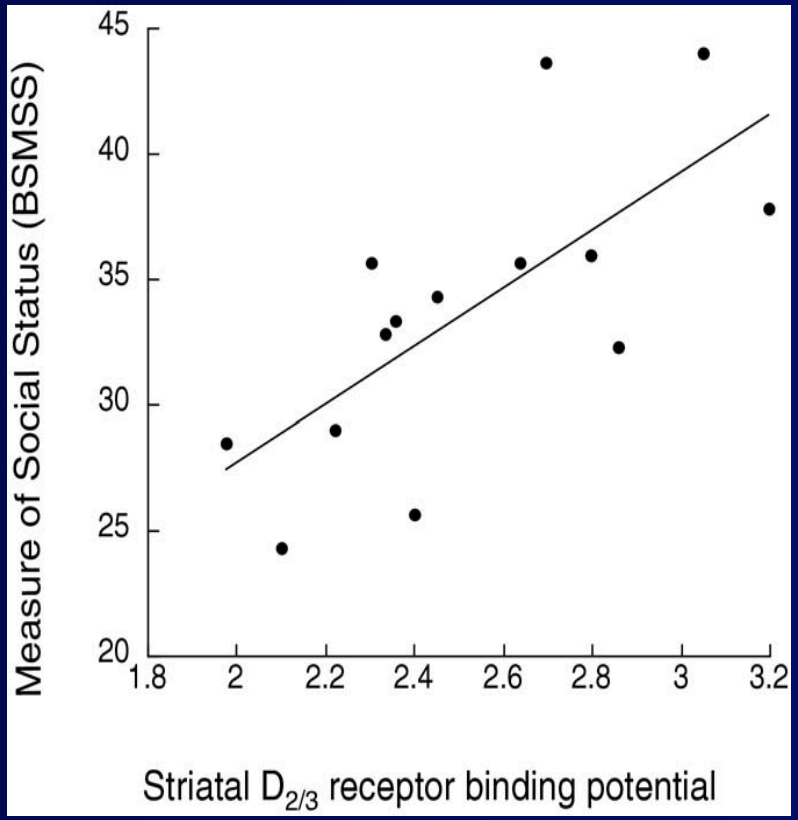
- Consistent with rat studies showing…
  - ↑ density of DA receptors in enriched
  - ↑ DA neurotransmission in isolation - DA hyperactivity

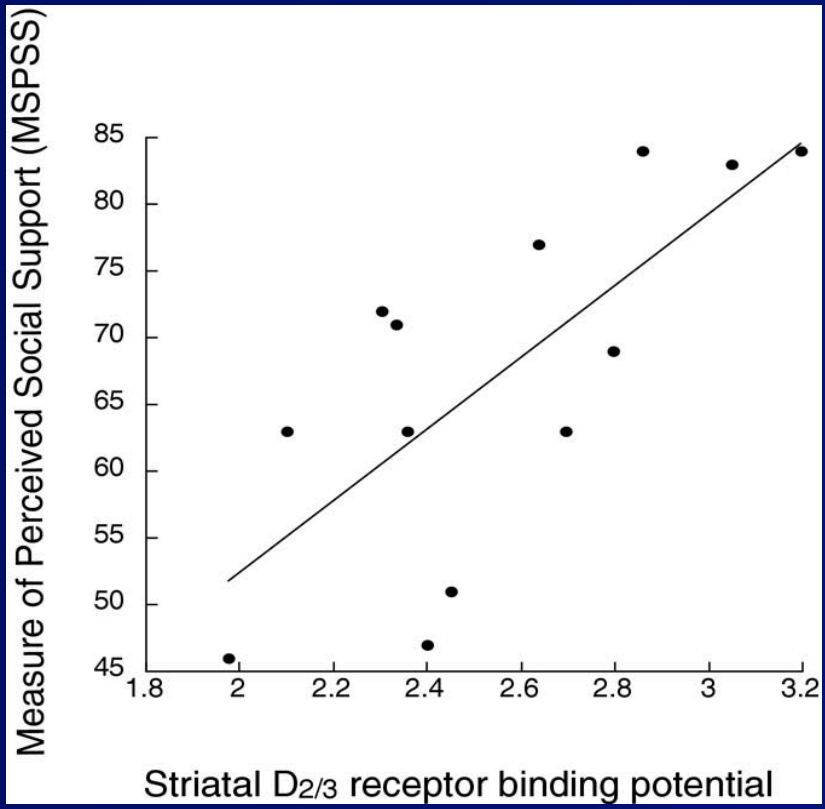
SES effect?

BRIEF REPORTS

Dopamine Type 2/3 Receptor Availability in the Striatum and Social Status in Human Volunteers

Diana Martinez, Daria Orlowska, Rajesh Narendran, Mark Slifstein, Fei Liu, Dileep Kumar, Allegra Broft, Ronald Van Heertum, and Herbert D. Kleber

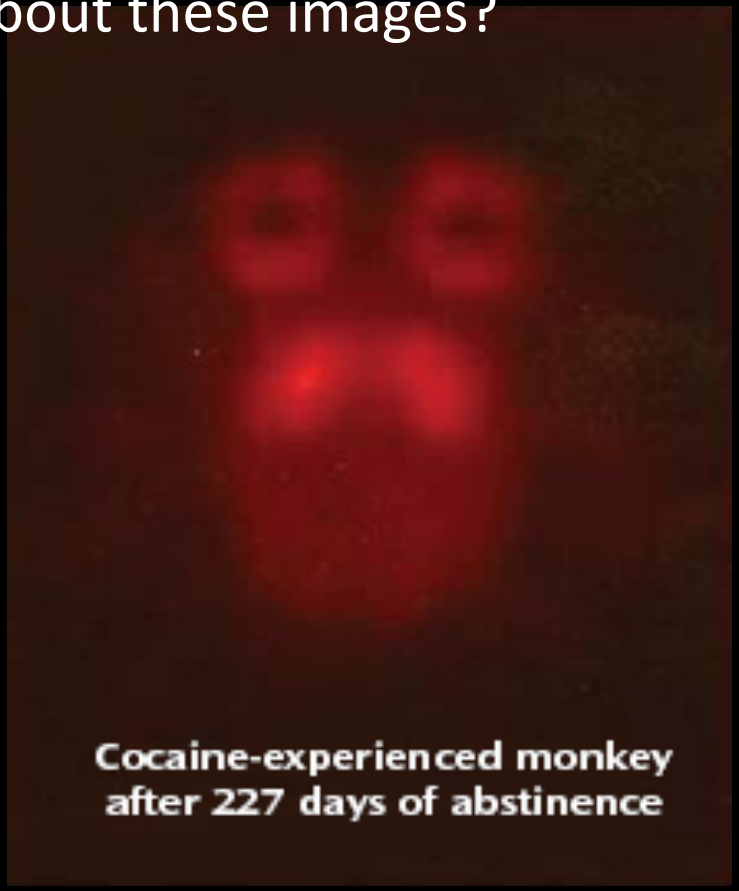




Monkeys who self-administered cocaine evidenced a profound long-lasting downregulation of the D2 receptor, subordinate = dominant; chronic cocaine leveled the playing field

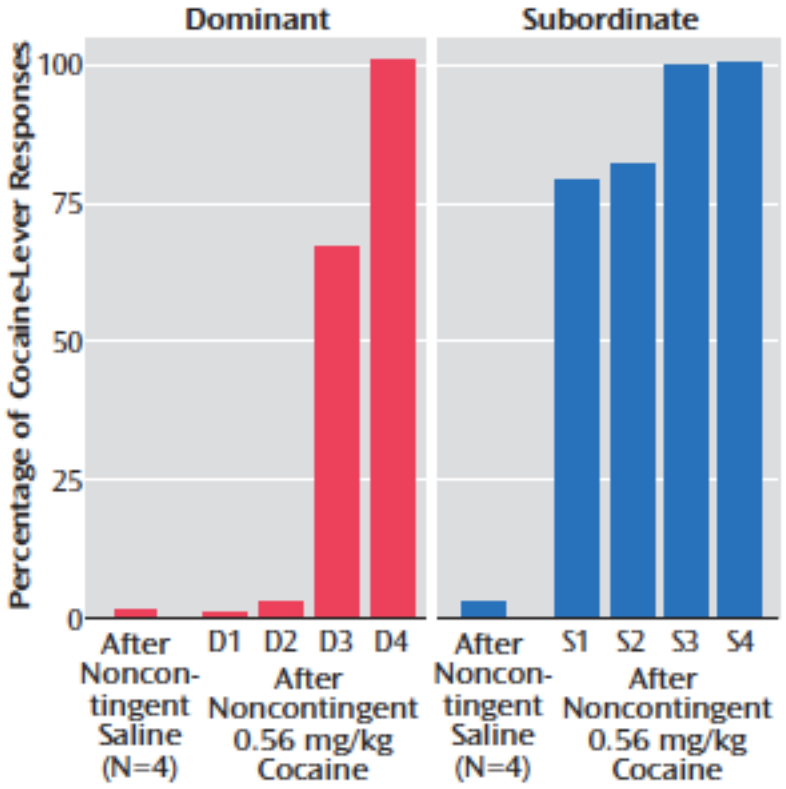
D2 receptor availability with other drugs???

What do we assume about these images?



Reinstatement

FIGURE 4. Effect of a Noncontingent Cocaine Injection on Individual Dominant (D1–D4) and Subordinate (S1–S4) Monkeys' Responses on a Lever Previously Associated With Cocaine but Currently Producing Saline Injections[a]



[a] The first bar for each group represents the average injection-lever responding following noncontingent saline injection. A significant difference was observed after noncontingent injection of 0.56 mg/kg cocaine between the dominant and subordinate monkeys. The data are from an unpublished study by P.W. Czoty, C. McCabe, and M.A. Nader.

Additional Questions/Talking Points

- DVR vs. Binding potential
- Hierarchy of animals might change, even during the course of the experiment.
- Effect of cocaine administered before social housing (during individual housing) ?
- Cocaine-self administration in Monkeys
- The authors cite some precious studies and say Hyper-active dopamine system is characteristic of Drug-prone phenotype. How do we define a hyperactive dopamine system? How can DVR or binding potential be related to a hyperactive dopamine system?
- What is the reinforcing effects of cocaine?
- What is the main reason for choosing particular PET agent for an experiment? If [11C]Rac was chose as the agent, would the result be the same as presented?
- What is the role of cocaine to monkeys? Do they know what cocaine is? Isn't cocaine used just as a tool for the reward?
- Is the level of DA decreased or D2R increased? Even though it is not clear however, authors chose to conclude that the availability of D2R increased, why?
- One large difference between dominant and subordinate primates is their plasma level of corticosterone. Does administering high levels of corticosterone or low levels (let's say after an adrenalectomy) modify dopamine in the same way?
- What about other types of reinforces, such as food? Do they find similar effects? Or other drugs that do not directly effect the dopaminergic system?

Additional Questions/Talking Points

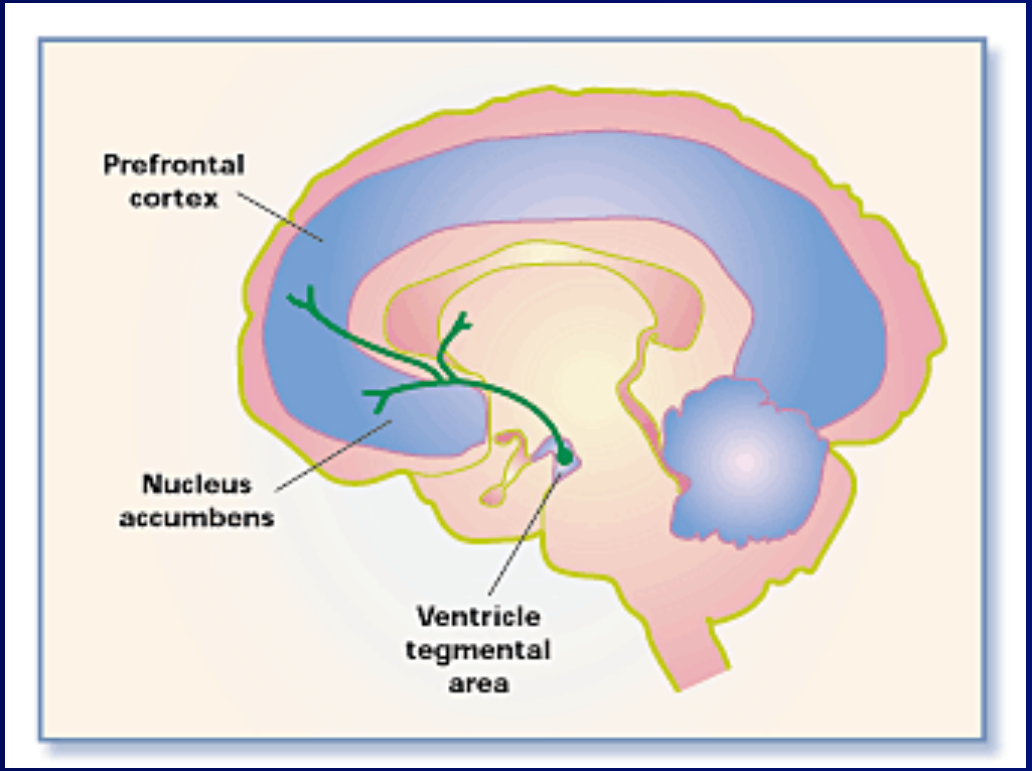
- Susceptibility to alcohol or other drugs across social ranks.
- Increased D2 receptors or decreased dopamine
- What if you returned the dominant monkeys to isolation, then ran PET scan again?
- What is an isocontour region?

Isocontour – contour of equal height? All the same?

- Are differences seen between dominant and subordinate monkeys in D2 and cocaine self-administration when socially housed from the start?
- What are the advantages/disadvantages of [^{18}F]FCP over [^{11}C]raclopride?

Nicotine

- Nicotine binds to nAChRs (alpha4beta2) on DA neurons in VTA, cell fires and releases DA in striatum.



What was the design of the Le Foll paper??

- Infusion
- BP_{ND} - specific binding / nondisplaceable uptake
- How did they pick the nicotine dose?

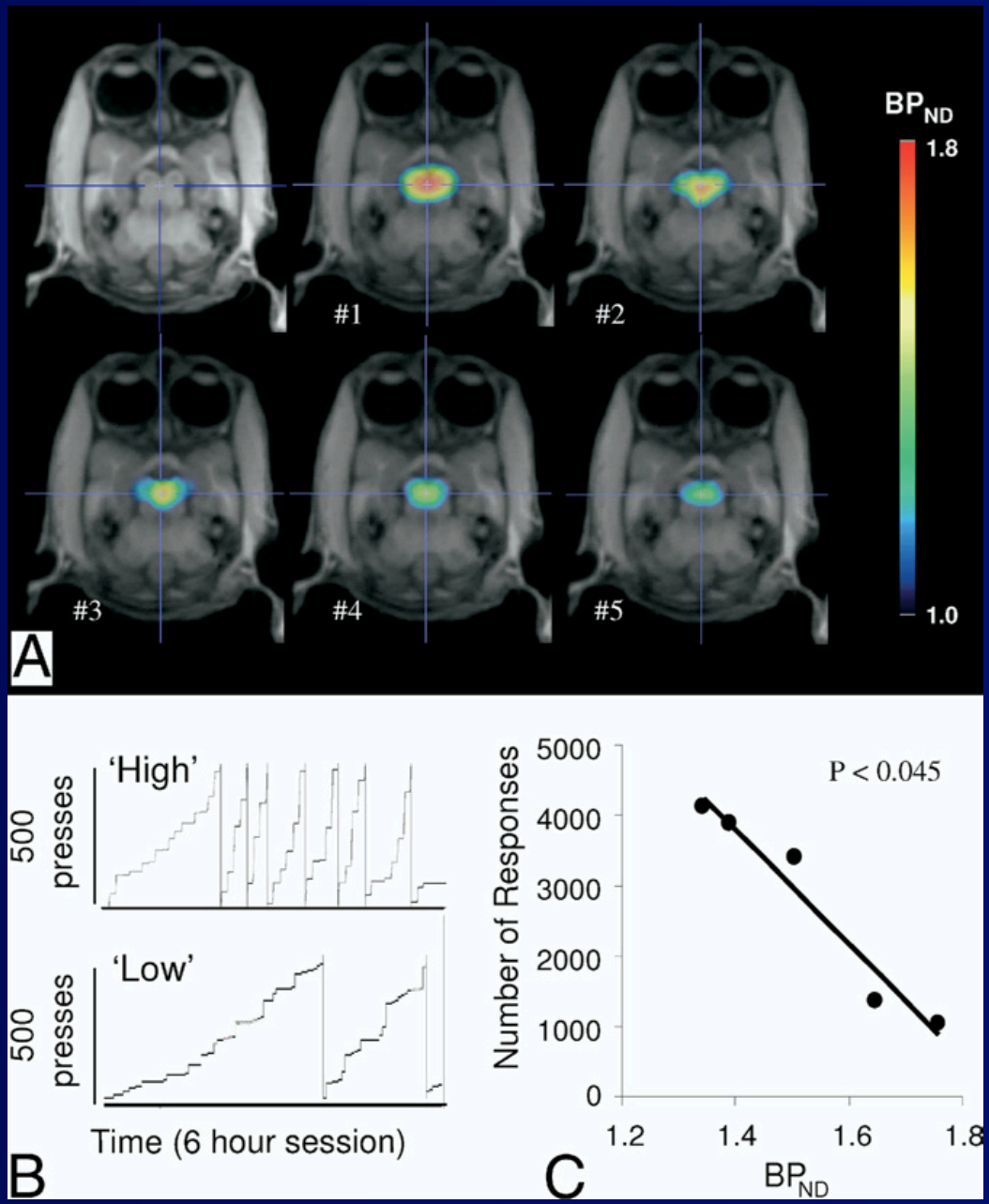
Reference region…

- **from the 1997 LeFoll JNM paper that they cite**

- **"The results of the present study demonstrate that 2-FA can accurately quantify nAChRs with a reference region outside the brain. These results suggest that the accurate quantification of central receptors is feasible with extracerebral reference regions, providing a novel approach for the quantification of brain receptors when no suitable brain reference region is available or ***when the assessment of the free fraction of the parent compound in the blood is difficult.*****

Talking points/Questions

- Reasons for the search for extracerebral regions or disadvantages/problems in arterial samplings and cerebral reference regions
- In page 1498 just below the picture, there's a mention of bias that might be introduced when using cerebellum as the reference. Is this pointing the bias between species? It will not be a good idea to use this model in humans anyways because the authors say that the human cerebellu has more specific receptors for 2-FA.
- Flipsides of using an extracerebral region like muscle, ex- effect of blood flow or activity.
- Questions:
- The way the authors have described the term ' α ', any region in the body, can be used as a reference provided we calculate a parallel term for α. I think the challenge (and my question) is how do we know if the α value will be constant and how reliable it is.



Le Foll et al., 2009

Talking points/Questions

- **PR-schedule (Correlation observed) vs. FR schedule (Correlation not observed)**
- **Number of animals too small to establish any significant relationship baseline receptor concentrations and their vulnerability to addiction.**
- **Since the previously conducted animal studies predict the opposite result, it might be possible that the obtained result was because of improper selection of ROI. A different region in the brain might have the same receptors and might be responsible for the change in motivation to self administer.**
- ***Midbrain = substantia nigra – full of dopamine – motivation – projections to striatum***
- **The genetically modified animal studies are establishing a cause-effect relationship while this study, even if validated with a large sample size, will at the maximum establish a correlation. Then, why is this study important?**
- **What is the reinforcing effects of nicotine?**
- **Why the studies in humans not ethical??**
- **What are the fixed-ration (FR) schedule of reinforcement and progressive-ratio schedule? When are they used?**
- **What are the motivational effects of nicotine?**
- Could they do corollary studies in mice with either viral vector knocking down or use a mutant to overexpress this specific nAChR in midbrain to look at nicotine self -administration? This could allow for an increased n and a more controlled experiment.
- Their N is very low.
- What about group housed?

Talking points/Questions

- **How do you control levels of varying levels of endogenous ligand due to changes in state of the monkeys under anesthesia?**
- **How do they define "low levels" of these nAchR subunits?**
- **These baseline levels could be influenced by/due to experiences each individual monkey had before the PET scan**
- **I would like to know how the monkeys who reached FR-10 the quickest did on the Progressive ratio task (I would think they would be the ones to push the lever the most during the 6 hrs and have the lowest baseline levels of midbrain alpha4beta2nAChR's…OR the opposite since the ones that took the longest to reach FR-10 might have been overtrained, made habitual prior to progressive ratio task and therefore pushed the level significantly more times and skewed the data b/c they were the ones who had the lowest levels of baseline binding)**
- **Explain why they also used muscle as ref region**
- **Doesn't anesthesia affect Ach levels?**

Additional questions/talking points

- **The authors suggest early that studies to assess the relation of baseline midbrain nAChRs to self-administration of nicotine would be unethical to conduct with humans. Can we think of studies that can address the overarching hypothesis that would be ethical in humans? For instance, it seems feasible to image baseline nAChRs levels, measure motivation/incentive salience, and then follow nicotine use longitudinally. Would such a study not adequately address the authors main hypothesis?**

- **Is nondisplaceable binding potential different from BP as we've learned it thus far? If so, how?**

Nomenclature paper – types of BP

- **Can we clarify the methods of intravenous nicotine self-administration? First, how does a fixed ratio differ from a progressive ratio schedule of reinforcement? How did the authors arrive at 30 ug/kg per injection dose of nicotine? Had it already been determined by previous literature that this was the optimal dose, or did they have to infer it from their own data? Also, what are "peak breaking point values?"**

30ug/kg = .03 mg/kg ; 1 cig = 1 mg/70 kg = .014 mg/kg and they cite a previous study where they probably did dose-repose studies

More…..

- **Why were both cerebellum and muscle tissue used as reference regions? That is, if there is nicotinic receptor expression in the cerebellum (as opposed to the complete absence of DA receptors there), why is that region used as a reference region at all?**

- **Figure 1B: My reading of these graphs is that a highly motivated monkey will very quickly arrive at a maximum number of "presses", and will quickly and repeatedly return to that level throughout the session, whereas a low motivated monkey will take longer to reach maximum presses, and will not as quickly or frequently return to that level over time. Is this a correct reading, or overly simplistic?**

- **Is there a good theoretical reason for the thalamus apparently not playing a role in nicotinic reinforcement despite the large number of receptors there?**

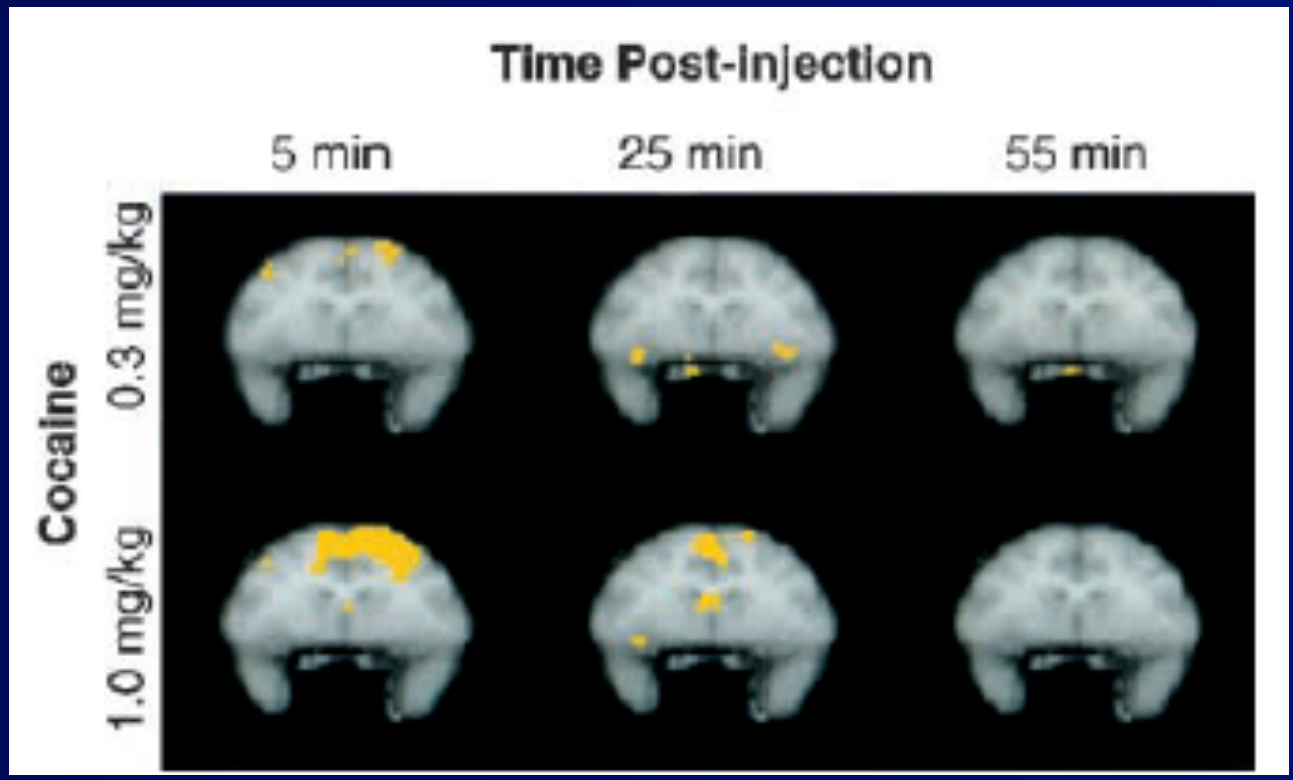
Midbrain = substantia nigra – full of dopamine – motivation – projections to striatum

More….

- **The principal finding/message of this study is that a low number of receptor availability is associated with high motivation to self-administer nicotine. Can we dissect this logic? I think the reasoning is that the occupation of receptors by endogenous acetylcholine/nicotine produces a rewarding effect that causes the subject to seek exogenous stimulation to continue to flood the receptors with nicotine. On the other hand, if the receptors are largely unoccupied by endogenous acetylcholine/nicotine, then the subject is less accustomed (right word?) to the sensation of nicotine-mediated reward (or is it less rewarded by nicotine?) and, therefore, less prone to seek exogenous stimulation. Perhaps we could wrestle with this line of reasoning.**

^{15}O water and PET blood flow imaging

IV cocaine in 4 conscious *drug naïve* monkeys leads to activation of the dorsolateral prefrontal cortex



Howell, Psychopharm, 2002

“Blocking” Study

Alaproclate is a SSRI that blocked cocaine activation