Mystery Neurotransmitters

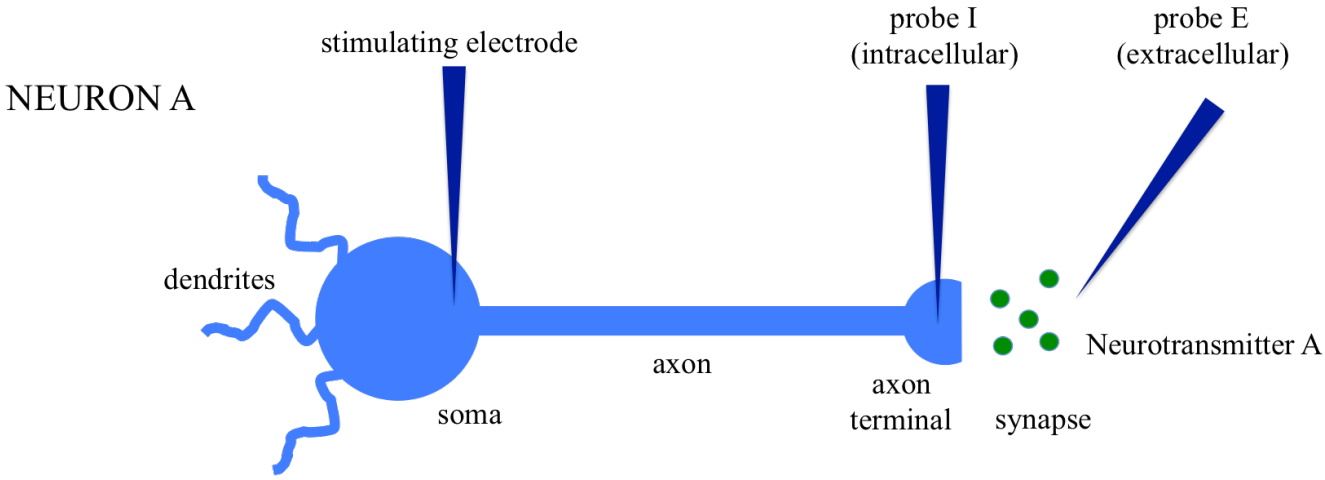
Neurons synthesize, package and release chemical messengers, called neurotransmitters, that bind to receptors on nearby neurons to directly open ion channel/receptor proteins or initiate second messenger signals and alter (e.g., increase or decrease) the neurons' electrical activity. A critical aspect of an effective chemical signal is the rapid termination of the signal, once its no longer needed. If a neurotransmitter stays in the synapse, it will continue to bind to available receptors on post-synaptic neurons. This can result in desensitization of the receptor so that it no longer functions. In these conditions, the receptor cannot convey time-sensitive information. Rapid termination of this signal ensures that the signal is time-limited and that additional signals can be sent soon afterward. Neurotransmitters can be cleared from the synapse by (a) reuptake into the pre-synaptic neuron, via specialized reuptake transporters, (b) enzymatic degradation, via specialized enzymes located in the synapse, and (c) passive diffusion (movement out of the synapse, driven by the concentration/chemical gradient). Diffusion occurs at all synapses; enzymatic degradation and reuptake may each be present at some synapses and not others.

In this activity, you will apply what you've learned about neurotransmitter clearance to the following problem set.

PART 1: Two unknown neurotransmitters

You are part of a research team of neuroscientists that studies chemical signaling in the brain, and how drugs of abuse can alter these signals. Your team has placed a set of small probes (yet to be invented!) into the brain of a mouse that is able to measure the intracellular and extracellular (synaptic) concentrations of neurotransmitters released by a particular neuron. And eureka! Your team discovers a neuron, "Neuron A," that releases an unknown neurotransmitter, "Neurotransmitter A." Your team wants to investigate what is happening to this mystery neurotransmitter at the synapse, after it has been released from Neuron A.

Your team lowers a stimulating electrode near the neuron's axon hillock and uses it to administer repetitive stimulus pulses, which initiates a series of action potentials. Then, your team records the intra- and extracellular concentrations of each neurotransmitter released into the synapse, using probes I and E.



Measurements from probes I and E are taken over 20 minutes. The action potential was initiated at time point 0. The data are graphed on the next page. Analyze these results and answer the subsequent questions.

Figure 1a

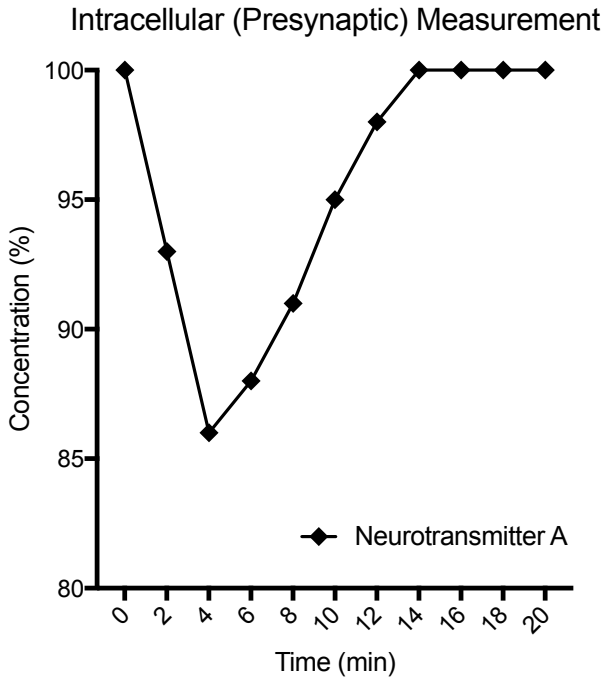
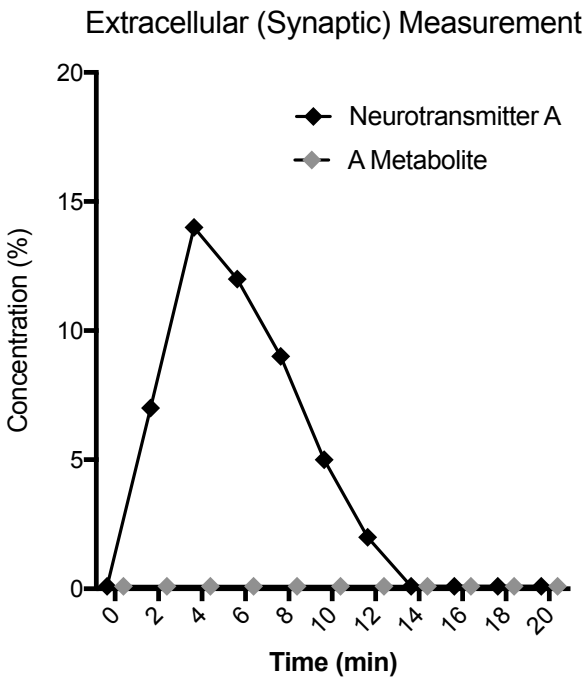


Figure 1b



1. What is the primary means by which Neurotransmitter A is removed from the synaptic cleft? Explain your answer – what evidence do you have?

A week later, your team discovers a second type of neuron, "Neuron B," that releases another unknown neurotransmitter, "Neurotransmitter B," using the same experimental setup as described above. Analyze the data graphed on the next page to answer the questions.

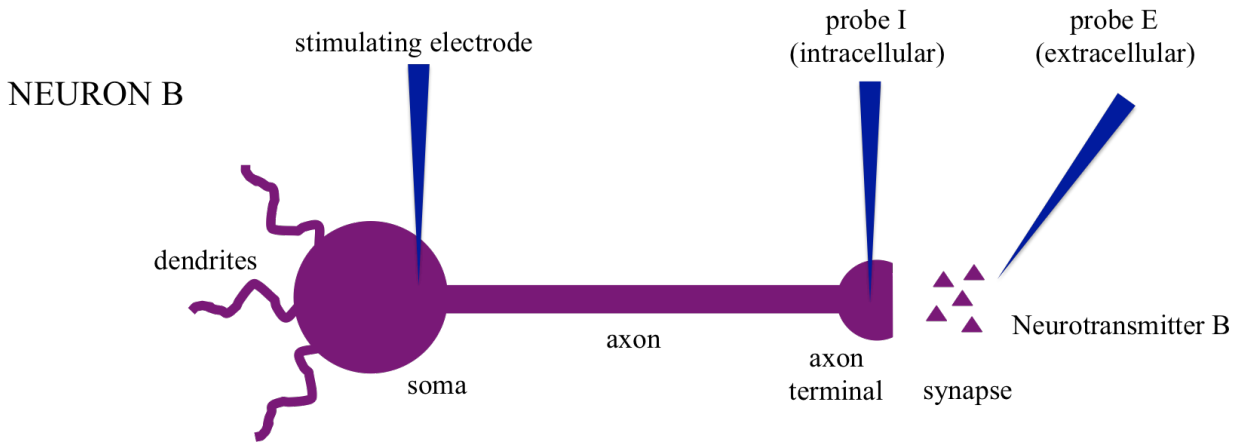


Figure 2a

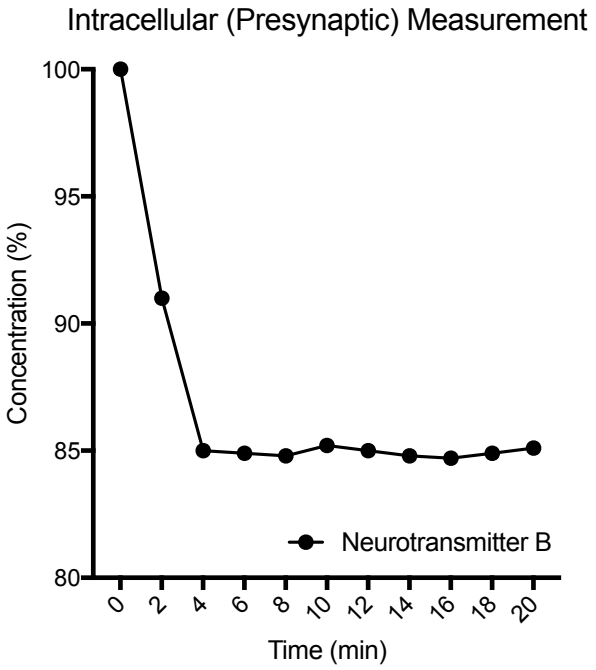
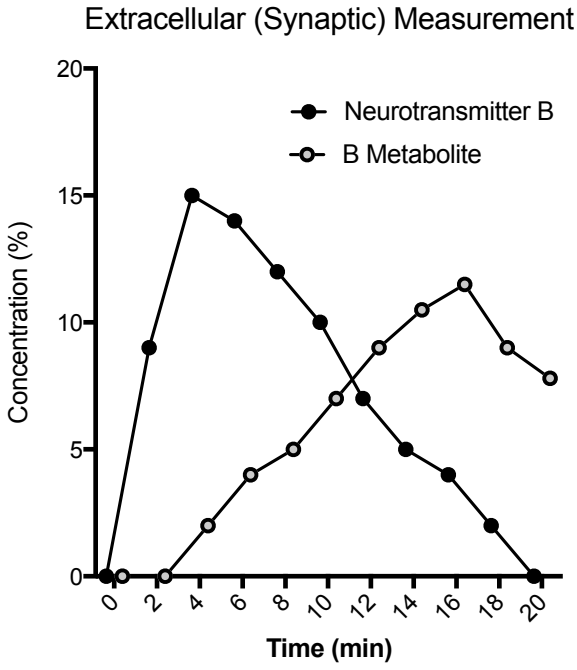


Figure 2b



2. What is the primary means by which Neurotransmitter B is removed from the synaptic cleft? Explain your answer – what evidence do you have?

3. Could another/secondary mechanism be contributing to clearance of Neurotransmitter B? If so, what might it be? What evidence might you need (e.g., what additional data might be useful or what kind of study might need to be performed) to suggest that another mechanism, besides the one in your answer above, is indeed contributing to clearance?

4. Was Neurotransmitter A or B removed from the synaptic cleft more rapidly? Explain your answer. What might this suggest about the speed of different mechanisms used to clear neurotransmitter from the synapse?

5. What might happen if the mouse were given an injection of a drug that blocked all of the reuptake transporters on these two neurons? Sketch a graph of the intra- and extracellular concentrations of Neurotransmitter A and B in the presence of a reuptake transporter inhibitor, on each of the graphs below. You do <u>not</u> need to include their metabolites. Just go for the general shape of the data; the overall patterns are most important.

Figure 3a

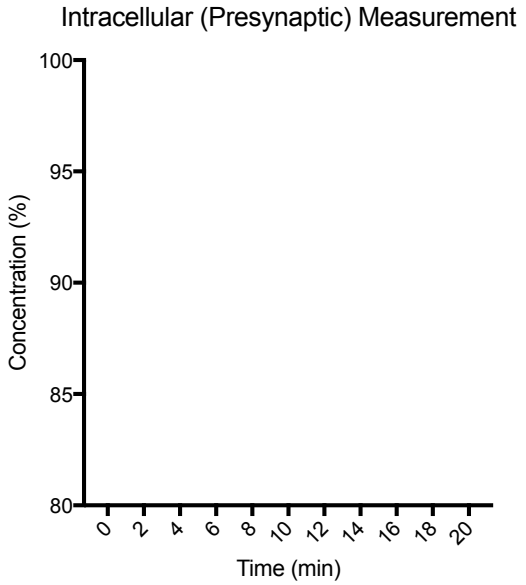
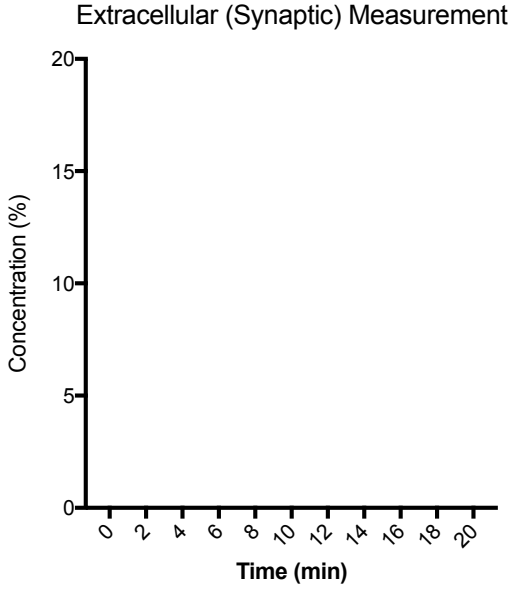


Figure 3b



6. In real life scenarios, clearance is determined, in part, by the transporters' density, distribution, and binding rate. How do you think that a higher density of transporters might affect the neurotransmitter's clearance rate?

PART 2: A new street drug

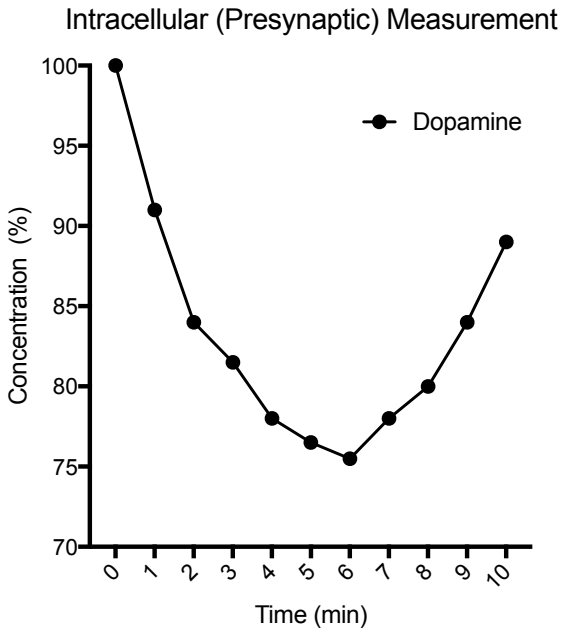
Two new street drugs have been growing in popularity in your community. Both drugs are abused recreationally and, with long-term use, seem to elicit strong cravings, suggesting that they may be addictive. According to local health officials, people who have taken these drugs report that they feel high and full of energy. Based on these clinical symptoms, your team speculates that these drugs are affecting dopamine neurotransmission.

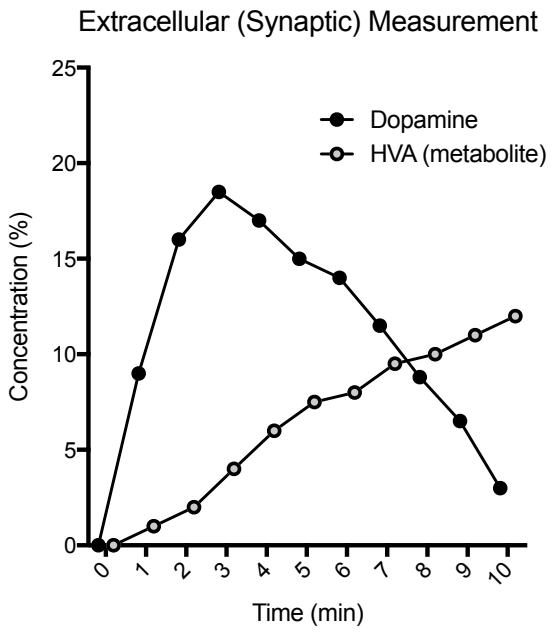
Dopamine (DA) is a catecholamine molecule that acts as a chemical signal at the synapse. Dopaminergic (dopamine-containing) neurons are located in two midbrain structures: the ventral tegmental area (VTA) and the substantia nigra (SN). These neurons project to a number of different brain regions. One subset of these DA neurons project from the VTA to the nucleus accumbens (NAc); this pathway, known as the mesolimbic DA system, is involved in reward- and motivation-related processes (e.g., Salamone & Correa 2012; Berridge, 2012; Salamone et al., 2018). Extracellular dopamine levels in the NAc change in response to the presentation of a variety of rewarding and aversive stimuli (e.g., Joseph et al., 2003; Young, 2004; Salamone et al., 2015). As many abused drugs act on the mesolimbic dopamine system (e.g., Di Chiara & Imperato 1988), you suspect that these two new street drugs may be acting on dopaminergic synapses.

The local police force has confiscated samples of these two drugs during their recent patrols, and has asked for your help analyzing them. Your team uses similar techniques to those used in Part 1. To gather baseline data, you first must determine how dopamine is cleared from the synapse.

Figure 4a

Figure 4b



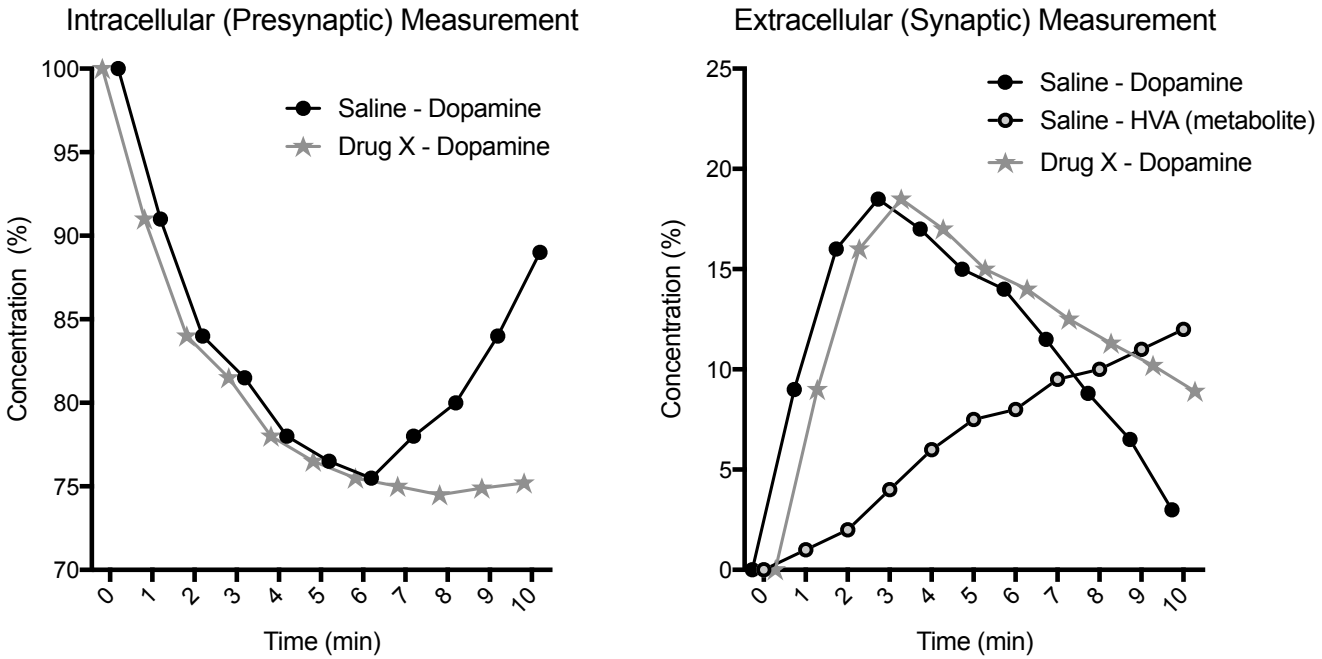


1. Mechanism(s) of dopamine clearance from the synapse:

On the next day, your team injects different sets of mice with either Drug X or saline, prior to stimulating the dopamine neuron. The data in Figure 5a, below, depict *presynaptic dopamine levels in the presence of Drug X or saline*. The data in Figure 5b, below, depict *extracellular dopamine levels and levels of its metabolite, HVA, in the presence of Drug X or saline*. Remember, only dopamine is being measured here; the street drugs just affect how dopamine acts at the synapse.

Figure 5a

Figure 5b



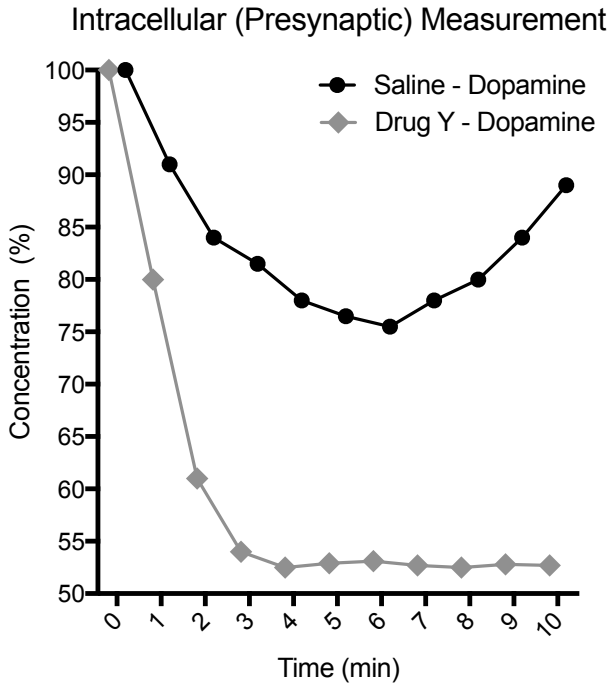
2. How does Drug X appear to change dopamine's storage, release and/or clearance from the synaptic cleft? Explain any/all plausible mechanisms.

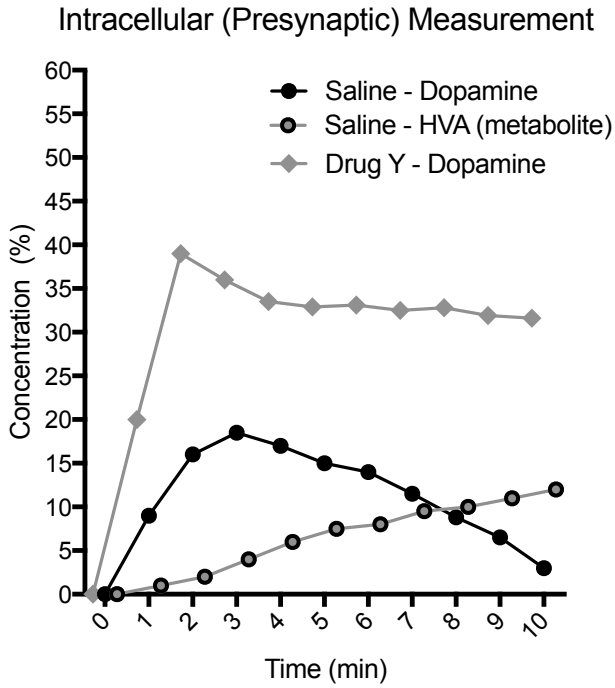
3. Given your answer, above, sketch a line depicting levels of HVA (a metabolite of dopamine) in the presence of Drug X onto graph B. This only needs to be a prediction – it doesn't have to be perfect. What factors did you take into account when sketching this line?

On the final day of your experiment, your team injects different sets of mice with either Drug Y or saline, prior to stimulating the dopamine neuron. The data in Figure 6a, below, depict *dopamine in the presence of Drug Y or saline*. The data in Figure 6b depict *dopamine and HVA in the presence of Drug Y or saline*. Note that the scale on the Y-axis has changed.

Figure 6a

Figure 6b





4. How does Drug Y appear to change dopamine's storage, release and/or clearance from the synaptic cleft? Explain any/all plausible mechanisms.

5. Given your answer, above, sketch a line depicting HVA levels in the presence of Drug Y onto graph B. What factor(s) did you take into account when sketching this line?

6. Which novel drug, X or Y, seems potentially more dangerous? Why?

7. Consider what else you'd like to know about these drugs. If you could design a follow-up study, what might it be? Come up with a simple experiment and hypothesis about what you'd predict that you'd see.

Select references:

Berridge KC (2012) From prediction error to incentive salience: Mesolimbic computation of reward motivation. Eur J Neurosci 35: 1124-1143. doi:10.1111/j.1460-9568.2012.07990.x

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA 85: 5274-5278.

Joseph MH, Datla K, Young AM (2003) The interpretation of the measurement of nucleus accumbens dopamine by in vivo dialysis: The kick, the craving or the cognition? Neurosci Biobehav Rev 27(6): 527-541.

Salamone JD, Correa M (2012) The mysterious motivational functions of mesolimbic dopamine. Neuron 76(3): 470-485. doi: 10.1016/j.neuron.2012.10.021.

Salamone JD, Pardo M, Yohn SE., López-Cruz L, SanMiguel N, Correa M (2015) Mesolimbic dopamine and the regulation of motivated behavior. In: Simpson E, Balsam P (eds) Behavioral Neuroscience of Motivation. Current Topics in Behavioral Neurosciences 27: 231-257. Springer, Cham

Salamone JD, Correa M, Yang JH, Rotolo R, Presby R (2018) Dopamine, effort-based choice, and behavioral economics: Basic and translational research. Front Behav Neurosci 12: 52. doi: 10.3389/fnbeh.2018.00052

Young AM (2004) Increased extracellular dopamine in nucleus accumbens in response to unconditioned and conditioned aversive stimuli: Studies using 1 min microdialysis in rats. J Neurosci Methods 138(1-2): 57-63.