PSYC4481: RESEARCH PRACTICUM IN BEHAVIORAL NEUROSCIENCE
Image Analysis Lab

Name: _____

CD #: _____

OBJECTIVE:
Gain experience analyzing images from real experiments for Fos-induction in: 1. a specific neuronal population and 2. a specific brain region. Compile data as a class. Discuss experimental design and results.

NOTE:
Do not copy or keep images. DISCS MUST BE RETURNED! Turn in packet at end of class.

DATA SUMMARY:
Please fill out this section once you complete your counts. This will be used by the TF to compile class data to run stats/make graphs.

Experiment 1: LHA (ORX+Fos)

Brain ID	Left Side						Right Side					
	Medial		Perifornical		Lateral		Medial		Perifornical		Lateral	
	#ORX	#Dbl	#ORX	#Dbl	#ORX	#Dbl	#ORX	#Dbl	#ORX	#Dbl	#ORX	#Dbl

Experiment 2: BLA (Fos)

Brain ID	**Level 26**		**Level 27**	
	Left Side	*Right Side*	*Left Side*	*Right Side*

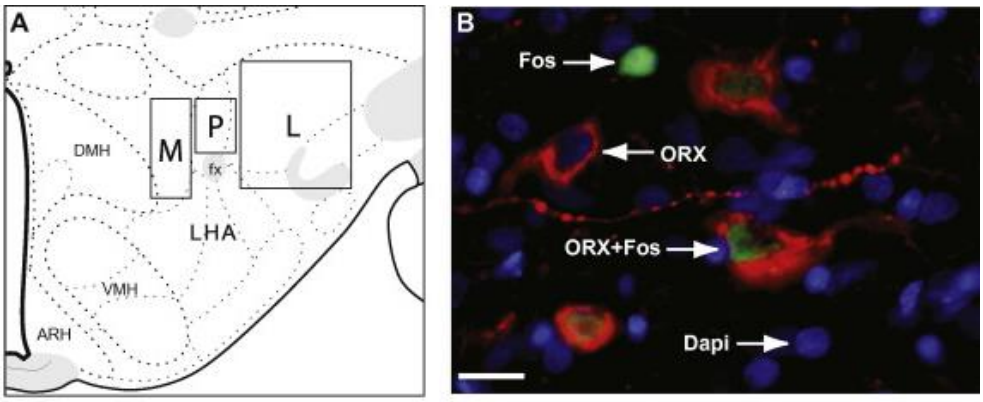
Experiment 3: CEA (Fos)

Brain ID	**Left Side**			**Right Side**		
	CEAl	*CEAm*	*CEAc*	*CEAl*	*CEAm*	*CEAc*

EXPERIMENT 1: Recruitment of Orexin Neurons During Cue-Induced Eating

In this study male rats received 10 training sessions each ~32 min long. For half of the rats (conditioned group, "Paired"), these sessions consisted of eight presentations of a 10 sec tone, each of which were immediately followed by delivery of food pellets into a food cup. For the other half of the rats (control group, "Unpaired"), the sessions consisted of the same number of tone and food presentations as the Paired group, but delivered in a non-conditional random order.

Two weeks later, all of the rats received 10 presentations of the tone over the course of five minutes. Rats were then left undisturbed for 75 min, at which time they were sacrificed. For visualization of Fos induction in orexin neurons, one series of brain tissues underwent two-step fluorescent double immunohistochemical processing for combined identification of orexin and Fos. Images of the tissue were taken bilaterally for three different locations within the lateral hypothalamus: directly above fornix (P), medial to fornix (M), and lateral to fornix (L).



We are interested in to what extent orexin neurons were engaged when rats heard the tone. To investigate this, you will be counting the total number of orexin-positive neurons and the number of double-labeled (orexin-positive + Fos-positive) neurons.

Independent Variable: _____

Dependent Variable: _____

Between-Subjects Factor: _____

Within-Subjects Factor: _____

Hypothesis:

EXPERIMENT 1: LHA Counting Instructions
File name code: Brain ID_Side_ Position_Region_Stain, e.g. KG22L 3,2 PF ORX+Fos+Dapi

1. Open ImageJ.

2. Go to "File" > "Import" > "Image Sequence. . ."

3. Navigate to the CD. Double-click on "Exp1-LHA". Double-click on one of the folders. Click to highlight the first image. Click "Open".

4. A dialogue box called "Sequence Options" will open. Do not edit, just click "OK".

5. Go to "Analyze" > "Tools" > "ROI Manager". Make sure the "Show All" box is checked.

6. Select the line tool. 

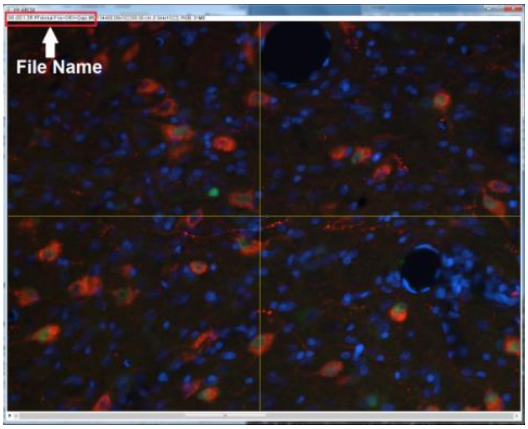
7. Draw a line across the ~horizontal center of the image. In the ROI Manager click "Add".

8. Draw a line across the ~vertical center of the image. In the ROI Manager click "Add".

9. Count the total # of orexin-positive neurons, and the # of those which are double-labeled for Fos in each quadrant.

    - Determining orexin-positive neurons:
        - Red cytoplasmic stain.
        - Neurons are counted as orexin-positive only if both their cell bodies & nuclei are visible.

    - Determining double-labeled neurons:
        - Red cytoplasmic stain with distinct green nuclei stain in center.



10. Record your counts in the tables on the next page. Once all quadrants for one image have been counted sum them together to calculate the totals for that image.
    NOTE: There are 3 image per hemisphere; record data into appropriate table.

11. Scrolling up/down with your mouse will take you to the next image. Repeat steps 9 & 10 until all images have been counted. Record final results in the table on page 1.

EXPERIMENT 1: LHA raw data

Brain ID: _____

Left Side

Medial		Perifornical		Lateral	
#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:
#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:
Total# ORX: _____ Total# DBL: _____		Total# ORX: _____ Total# DBL: _____		Total# ORX: _____ Total# DBL: _____	

Right Side

Medial		Perifornical		Lateral	
#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:
#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:
Total# ORX: _____ Total# DBL: _____		Total# ORX: _____ Total# DBL: _____		Total# ORX: _____ Total# DBL: _____	

Brain ID: _____

Left Side

Medial		Perifornical		Lateral	
#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:
#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:
Total# ORX: _____ Total# DBL: _____		Total# ORX: _____ Total# DBL: _____		Total# ORX: _____ Total# DBL: _____	

Right Side

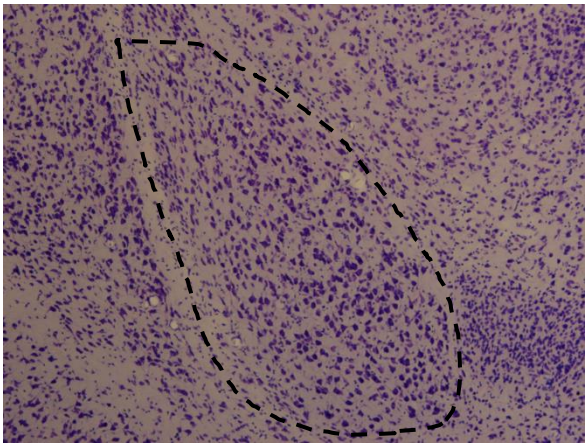
Medial		Perifornical		Lateral	
#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:
#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:	#ORX: #Dbl:
Total# ORX: _____ Total# DBL: _____		Total# ORX: _____ Total# DBL: _____		Total# ORX: _____ Total# DBL: _____	

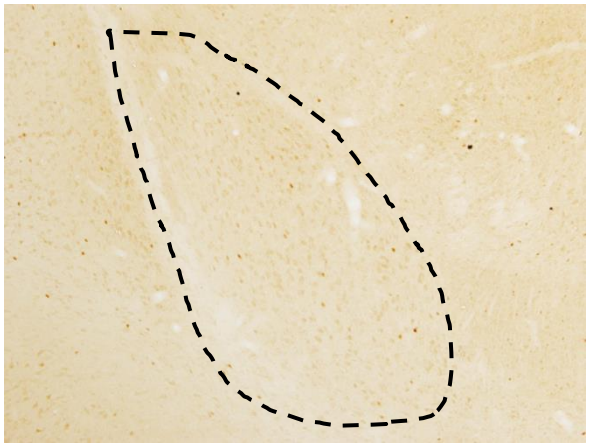
EXPERIMENT 2: Recruitment of Basolateral Amygdala across Pavlovian Conditioning

In this study male rats received 1 or 10 ~32min training sessions. For half of the rats (conditioned group, "Paired"), these training sessions consisted of eight presentations of a 10 sec tone, each of which were immediately followed by delivery of food pellets into a food cup. For the other half of the rats (control group, "Tone"), the sessions consisted of the same number of tone presentations as the Paired group but no food was delivered.

Depending on group assignment (Day 1 or Day 10), on their last training day rats were returned to their rooms and left undisturbed for 90min, at which time they were sacrificed. One series of brain tissue underwent a three-step peroxidase immunohistochemical processing for visualization of Fos-alone, and one series of brain tissue was stained using the nissl technique.

We are interested in to what extent the anterior basolateral amygdala (BLAa) is recruited during early learning (in the 1st training session) compared to late learning (after 10 training sessions). To investigate this, you will be using ImageJ to count the number of Fos-positive neurons in two atlas levels of the BLAa based on borders you draw on an adjacent nissl-stained section.





Independent Variable: _____

Dependent Variable: _____

Between-Subjects Factor: _____

Within-Subjects Factor: _____

Hypothesis:

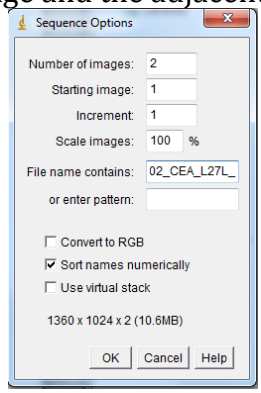
EXPERIMENT 2: BLA Fos Counting Instructions
File name code: Brain ID_Side_Region_Level_Stain, e.g. ACQ9 L BLAa L26 Fos

1. Open ImageJ.

2. Go to "File" > "Import" > "Image Sequence. . ."

3. Navigate to the CD. Double-click on "Exp2-BLA". Double-click on one of the folders. Click on one of the Fos images. Highlight the name up to, but not including "Fos". Hold Ctrl-C to copy. Click "Open".

4. A dialogue box called "Sequence Options" will open. Change "Number of Images" to 2. Click in the space next to "File name contains:". Hold Ctvl-V to paste. Click "OK". This will now import only your Fos image and the adjacent nissl image.



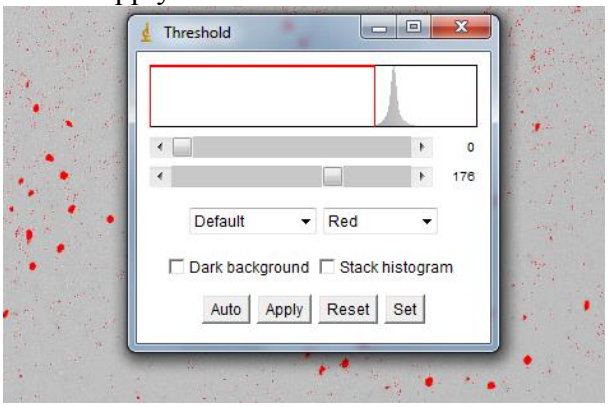
5. Select the polygon tool. 

6. Scroll to the nissl image. Using the polygon tool outline the BLAa.

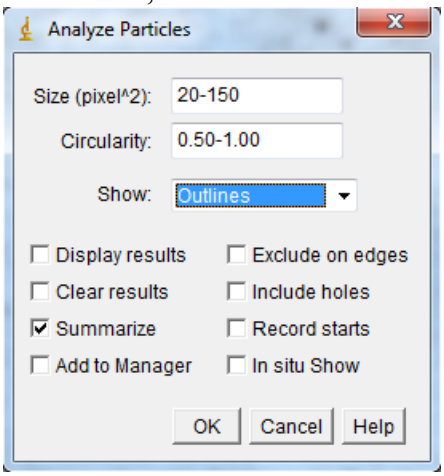
7. Go to "Edit" and choose "Clear Outside". Now all the remains in the BLAa.

8. Scroll to the Fos image. Go to "Image" > "Type" > "8-bit" to change image to B&W.

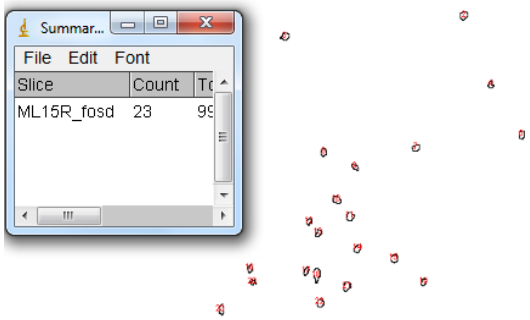
9. Go to "Image" > "Adjust" > "Threshold". Adjust bottom slider bar so that the Fos-positive neurons are filled in. Click "Apply".



10. Go to “Analyze” > “Analyze Particles”, and fill out the info as follows:

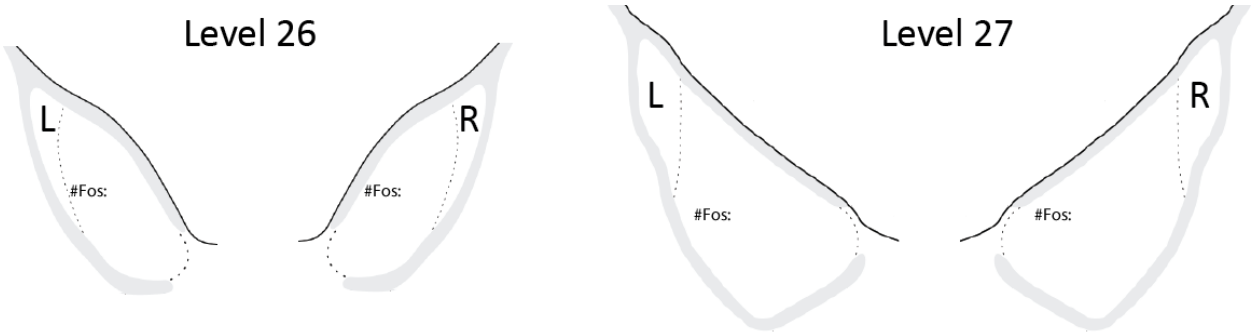


11. Two new windows will pop up: one with the results from the count and the other with all the neurons that were counted circled. Record the “Count” value in the appropriate place on the figures at the bottom of this page. Close these windows and your images (but not the program!).



12. Repeat steps 2-11 for the other image pairs. When done, transpose results to the appropriate table on Page 1.

Brain ID: _____

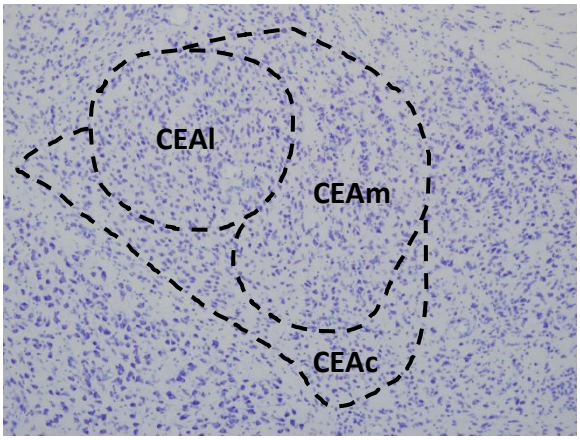


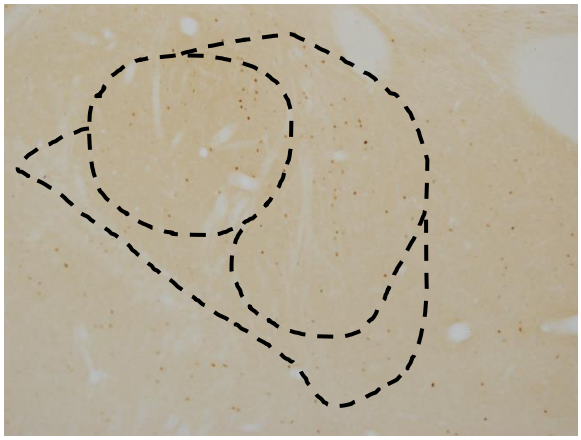
EXPERIMENT 3: Activation of Central Amygdala during Tone-Cue Inhibition of Eating

In this study male and female rats received two types of training sessions that occurred in two distinct contexts. During the "appetitive" sessions all rats were given food pellets. During the "aversive" sessions half of the male and female rats received 2 mild footshocks each signaled by a 60 sec tone (conditioned groups), while the other half of the rats received only the tones (control groups).

Following training completion, all rats received 4 presentations of the tone (no footshocks were delivered) during a 10min session in the appetitive context where they had the opportunity to eat. Rats were then returned to their rooms and left undisturbed for 90min, at which time they were sacrificed. One series of brain tissue underwent a three-step peroxidase immunohistochemical processing for visualization of Fos-alone, and one series of brain tissue was stained using the nissl technique.

We are interested in the extent to which the three divisions of central nucleus of the amydala (CEA) were engaged during the test session. To investigate this, you will be using ImageJ to count the number of Fos-positive neurons for one atlas level of the CEA based on borders you draw on an adjacent nissl-stained section.





Independent Variable: _____

Dependent Variable: _____

Between-Subjects Factor: _____

Within-Subjects Factor: _____

Hypothesis:

EXPERIMENT 3: CEA Counting Instructions
File name code: Brain ID_Region_Level&Side_Stain&Position, e.g. TI02_CEA_L27L_Fos9.4

1. Follow steps 1-5 from BLA counting, choosing pairs of images from "Exp3-CEA".

2. Go to "Analyze" > "Tools" > "ROI Manager". Make sure the "Show All" box is checked.

3. Scroll to the nissl image. Using the polygon tool outline one of the CEA subdivisions. In the ROI Manager click "Add". Click to highlight your addition in the ROI manager, then click "Rename" and name your region appropriately. Repeat for the other two subdivisions. Note: Each subdivision must be a complete polygon.

4. Follow steps 8 & 9 from BLA counting.

5. Highlight one of your subdivisions on the ROI Manager, then make sure you are scrolled to the Fos image. Go to "Analyze" > "Analyze Particles", and fill the info the same as you did for the BLA counting.

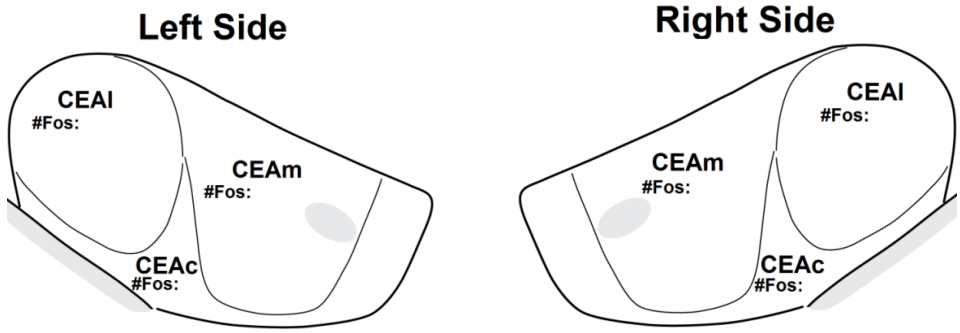
6. Record the "Count" value on the figure at the bottom of this page. Close the pop-up windows (but not your images or ImageJ!).

7. Repeat steps 5 & 6 for other subdivisions.

8. Now close your images, and repeat steps 2-7 for another image pair. When done, transpose results to appropriate table on Page 1.

Brain ID:

_____



Brain ID:

_____