Import all packages

In [1]:
```
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
import seaborn as sns
from datetime import datetime
%matplotlib inline
```

Stress Enhanced FearLearning - Freezing Analysis

Stress-enhanced fear learning (SEFL) is a Pavlovian behavioral paradigm used to model pathological anxiety and fear overgeneralization. The SEFL protocol was developed by the Fanselow group to recapitulate critical aspects of PTSD including long-term sensitization of fear learning caused by an acute stressor (Rau et al. 2005; Au - Rajbhandari et al 2018). On Day 1, mice receive 4 randomly-timed footshocks (1 mA, 2s, average ISI = 180 seconds) in Context A. On Day 2, mice are placed back in Context A, and freezing behavior in the aversive context is measured for 5 minutes. On Day 3, mice are placed in a separate Context B and delivered a mild stressor consisting of a novel tone given after a 180-s baseline period. The "trauma" procedure on Day 1 in Context A will enhance the fear response for the mild stressor administered in Context B. Mice show minimal fear generalization from the traumatic context but show increased fear response in the context where the mild stressor was administered.

In these experiments, we are testing whether previous experience of early life stress (ELS), using the limited bedding and nesting (LBN) model, affects the development of persistent fear overgeneralization. Animals reared under ELS or control (Ctrl) conditions will be assessed for their freezing during Context A training and testing as well as in Context B to a mild stressor.

The software used to assess the freezing behavior (Ethovision) measures the animals' time immobile across time bins for each arena and outputs an anonymized wide format data frame. We will convert this into long format and merge with metadata about each individual for subsequent group comparisons.

Convert dataframe from default Ethovision output

Import (default) ethovision export file from inactivity analysis

Based on the original structure of the data output, I import with multiindexing to allow for Arena and Freezing thresholds to become columns later on.

In [2]:
```
etho_df = pd.read_csv('SEFL_Inactivity_Cohort_1.csv',header = [3,4])
```

In [3]:
```
etho_df #examine data frame
```

Out[3]:

	Unnamed: 0_level_0	Unnamed: 1_level_0	Unnamed: 2_level_0	Arena 1	Arena 2	Arena 3	Arena 4	Arena 1	Arena 2	Arena 3	Arena 4	Arena 1	Arena 2	Arena 3	Arena 4
	Unnamed: 0_level_1	Unnamed: 1_level_1	Unnamed: 2_level_1	.10freeze	.10freeze	.10freeze	.10freeze	.05freeze	.05freeze	.05freeze	.05freeze	.03freeze	.03freeze	.03freeze	.03freeze
0	Trial 1	ContextAtraining	0:00:00-0:00:30	2.12	6.00	5.04	5.68	0.96	2.08	3.40	3.60	0.16	0.80	2.76	2.28
1	Trial 1	ContextAtraining	0:00:30-0:01:00	2.96	6.28	10.84	8.48	1.24	3.80	8.44	5.52	0.64	2.28	6.60	3.48
2	Trial 1	ContextAtraining	0:01:00-0:01:30	4.80	3.24	7.40	10.56	3.04	1.08	3.48	7.56	1.80	0.64	2.32	5.72
3	Trial 1	ContextAtraining	0:01:30-0:02:00	10.28	1.68	10.20	11.44	7.16	0.24	5.56	8.20	5.68	0.16	3.32	6.36
4	Trial 1	ContextAtraining	0:02:00-0:02:30	6.60	2.96	15.08	1.40	3.56	1.64	8.96	0.72	2.40	0.84	6.36	0.36
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
377	Trial 39	ContextBTest	0:01:30-0:02:00	8.16	9.72	NaN	NaN	6.88	7.08	NaN	NaN	6.32	5.68	NaN	NaN
378	Trial 39	ContextBTest	0:02:00-0:02:30	18.16	7.36	NaN	NaN	15.48	3.52	NaN	NaN	13.56	2.52	NaN	NaN
379	Trial 39	ContextBTest	0:02:30-0:03:00	18.12	3.72	NaN	NaN	13.24	1.80	NaN	NaN	10.92	1.36	NaN	NaN
380	Trial 39	ContextBTest	0:03:00-0:03:30	11.04	7.44	NaN	NaN	8.08	5.72	NaN	NaN	7.04	4.72	NaN	NaN
381	Trial 39	ContextBTest	0:03:30-0:04:00	9.12	11.60	NaN	NaN	5.72	8.16	NaN	NaN	4.60	6.28	NaN	NaN

382 rows × 15 columns

Here, we rename the unnammed columns to something useful and discard the lower level

In [4]:
```
etho_df = etho_df.rename(columns={"Unnamed: 0_level_0": "Trial",
                                  "Unnamed: 1_level_0": "Experiment",
                                  "Unnamed: 2_level_0": "Time_Bin",
                                  "Unnamed: 0_level_1": None,
                                  "Unnamed: 1_level_1": None,
                                  "Unnamed: 2_level_1": None
                                 })
```

You'll see that there are 3 different freezing thresholds that have been collected. For the sake of simplicity, we will drop all but the most stringent setting `.03freeze`. (Would be nice for functionality to turn this into a function/selector for which freezing threshold using if/else)

In [5]:
```
etho_df_03 = etho_df.drop(columns = ['.10freeze', '.05freeze'], level = 1)
```

Now, we melt the table so that `Arena` becomes it's own column and the freezing duration is distributed between them for each animal

In [6]:
```
etho_melt = etho_df_03.melt(col_level=0,
    id_vars=['Trial', 'Experiment', 'Time_Bin'],#columns to keep
    var_name= 'Arena', #new column name
    value_name= 'freezing_duration(s)' #new column for freezing
)
```

Calculate Percent Freezing for each time bin

Here, we add a column for the length of each bout (30 seconds). Eventually would like to be able to derive this from the time stamp.

In [7]:
```
etho_melt.insert(5,"Bout(s)", 30) #same insert as above, adding to far right
```

Prior to running calculations on columns, verify the data types with `type` and `Loc`.

In [8]:
```
#veryifying data types prior to division
#type(etho_tidy.loc[0,'freezing duration (s)'])
#type(etho_tidy.loc[0,'Bout (s)'])
```

Add a new column called `% Freezing` that takes `freezing duration (s)` divided by `Bout (s)` and multiply by 100.

In [9]:
```
etho_melt["PcentFreezing"] = (etho_melt['freezing_duration(s)']/ etho_melt['Bout(s)'])*100
```

Using the `Time_Bin` column, generate a new column called `Time(s)` containing the time bin total in seconds with a `for` loop. Considered converting to timedelta but...did not work.

In [10]:
```
result = []
for string in etho_melt['Time_Bin']:
    if string == '0:00:00-0:00:30':
        result.append(30)
    elif string == '0:00:30-0:01:00':
        result.append(60)
    elif string == '0:01:00-0:01:30':
        result.append(90)
    elif string == '0:01:30-0:02:00':
        result.append(120)
    elif string == '0:02:00-0:02:30':
        result.append(150)
    elif string == '0:02:30-0:03:00':
        result.append(180)
    elif string == '0:03:00-0:03:30':
        result.append(210)
    elif string == '0:03:30-0:04:00':
        result.append(240)
    elif string == '0:04:00-0:04:30':
        result.append(270)
    elif string == '0:04:30-0:05:00':
        result.append(300)
    elif string == '0:05:00-0:05:30':
        result.append(330)
    elif string == '0:05:30-0:06:00':
        result.append(360)
    elif string == '0:06:00-0:06:30':
        result.append(390)
    elif string == '0:06:30-0:07:00':
        result.append(420)
    elif string == '0:07:00-0:07:30':
        result.append(450)
    else:
        result.append('NaN')

etho_melt['Time(s)'] = result
etho_melt
```

Out[10]:

	Trial	Experiment	Time_Bin	Arena	freezing_duration(s)	Bout(s)	PcentFreezing	Time(s)
0	Trial 1	ContextAtraining	0:00:00-0:00:30	Arena 1	0.16	30	0.533333	30
1	Trial 1	ContextAtraining	0:00:30-0:01:00	Arena 1	0.64	30	2.133333	60
2	Trial 1	ContextAtraining	0:01:00-0:01:30	Arena 1	1.80	30	6.000000	90
3	Trial 1	ContextAtraining	0:01:30-0:02:00	Arena 1	5.68	30	18.933333	120
4	Trial 1	ContextAtraining	0:02:00-0:02:30	Arena 1	2.40	30	8.000000	150
...	...	...	...	...	...	...	...	...
1523	Trial 39	ContextBTest	0:01:30-0:02:00	Arena 4	NaN	30	NaN	120
1524	Trial 39	ContextBTest	0:02:00-0:02:30	Arena 4	NaN	30	NaN	150
1525	Trial 39	ContextBTest	0:02:30-0:03:00	Arena 4	NaN	30	NaN	180
1526	Trial 39	ContextBTest	0:03:00-0:03:30	Arena 4	NaN	30	NaN	210
1527	Trial 39	ContextBTest	0:03:30-0:04:00	Arena 4	NaN	30	NaN	240

1528 rows × 8 columns

In a later step `merge`, we identified a couple issues with some `Trial` strings containing additional spaces. We remove them here using the `replace` function.

In [11]:
```
etho_shrink = etho_melt.replace('Trial     ', 'Trial ', regex=True) # replace 5 spaces with one, using regular expresion
etho_shrink2 = etho_shrink.replace('Trial    ', 'Trial ', regex=True) #replace the 4 additional spaces with 1
```

In [12]:
```
etho_shrink2['Trial'].iloc[0] # check if 5 space has changed
```

Out[12]:
```
'Trial 1'
```

In [13]:
```
etho_shrink2['Trial'].iloc[80] # check if  4 space has changed
```

Out[13]:
```
'Trial 6'
```

Several rows also contain NaN because they are empty arenas with out any freezing data, we drop those with `dropna`.

In [14]:
```
etho_tidy = etho_shrink2.dropna(axis = 'rows') #requires axis to keep in DataFrame format
etho_tidy
```

Out[14]:

	Trial	Experiment	Time_Bin	Arena	freezing_duration(s)	Bout(s)	PcentFreezing	Time(s)
0	Trial 1	ContextAtraining	0:00:00-0:00:30	Arena 1	0.16	30	0.533333	30
1	Trial 1	ContextAtraining	0:00:30-0:01:00	Arena 1	0.64	30	2.133333	60
2	Trial 1	ContextAtraining	0:01:00-0:01:30	Arena 1	1.80	30	6.000000	90
3	Trial 1	ContextAtraining	0:01:30-0:02:00	Arena 1	5.68	30	18.933333	120
4	Trial 1	ContextAtraining	0:02:00-0:02:30	Arena 1	2.40	30	8.000000	150
...	...	...	...	...	...	...	...	...
1427	Trial 27	ContextBTest	0:01:30-0:02:00	Arena 4	30.00	30	100.000000	120
1428	Trial 27	ContextBTest	0:02:00-0:02:30	Arena 4	30.00	30	100.000000	150
1429	Trial 27	ContextBTest	0:02:30-0:03:00	Arena 4	30.00	30	100.000000	180
1430	Trial 27	ContextBTest	0:03:00-0:03:30	Arena 4	30.00	30	100.000000	210
1431	Trial 27	ContextBTest	0:03:30-0:04:00	Arena 4	30.00	30	100.000000	240

1144 rows × 8 columns

Generating a Complete Dataset with Animal Info

Import metadata

In [15]:
```
etho_meta = pd.read_csv('SEFL_Cohort1_metadata.csv')
etho_meta
```

Out[15]:

	Cohort	Cage	Dam ID	DOB	Exp Start	Experiment	Mouse	Sex	Condition	Arena	Trial
0	1	119447	279_C_2	6/23/2022	10/4/2022	ContextAtraining	1	F	Ctrl	1	Trial 1
1	1	119447	279_C_2	6/23/2022	10/4/2022	ContextAtraining	2	F	Ctrl	2	Trial 1
2	1	119447	279_C_2	6/23/2022	10/4/2022	ContextAtraining	3	F	Ctrl	3	Trial 1
3	1	119447	279_C_2	6/23/2022	10/4/2022	ContextAtraining	4	F	Ctrl	4	Trial 1
4	1	119476	279_C_2	6/23/2022	10/4/2022	ContextAtraining	5	F	Ctrl	1	Trial 2
...	...	...	...	...	...	...	...	...	...	...	...
91	1	119470	237_E_3	6/26/2022	11/3/2022	ContextBTest	7	M	ELS	3	Trial 37
92	1	119470	237_E_3	6/26/2022	11/3/2022	ContextBTest	8	M	ELS	4	Trial 38
93	1	113342	265_E_5	6/28/2022	11/3/2022	ContextBTest	9	M	ELS	1	Trial 38
94	1	113342	265_E_5	6/28/2022	11/3/2022	ContextBTest	10	M	ELS	2	Trial 39
95	1	113342	265_E_5	6/28/2022	11/3/2022	ContextBTest	11	M	ELS	3	Trial 39

96 rows × 11 columns

Make Sure that Key Columns Match

Adding the word "Arena" to `Arena` string.

In [16]:
```
etho_meta.insert(9, "concat", "Arena") #add a new column called 'concat' with the str "Arena"
```

In [17]:
```
etho_meta['Arena'] = etho_meta[['concat', 'Arena']].astype(str).apply(" ".join, axis=1) #concatenate the two columns using apply function
```

In [18]:
```
etho_meta_mod = etho_meta.drop(columns='concat') # drop the unnecessary column
```

Generate an ID Column combining `Mouse` and `Sex`

In [19]:
```
etho_meta_mod['Mouse ID'] = etho_meta_mod[['Mouse ', 'Sex']].astype(str).apply("_".join, axis=1) #concatenate the two columns using apply functio
```

In [20]:
```
etho_meta_mod #check work
```

Out[20]:

	Cohort	Cage	Dam ID	DOB	Exp Start	Experiment	Mouse	Sex	Condition	Arena	Trial	Mouse ID
0	1	119447	279_C_2	6/23/2022	10/4/2022	ContextAtraining	1	F	Ctrl	Arena 1	Trial 1	1_F
1	1	119447	279_C_2	6/23/2022	10/4/2022	ContextAtraining	2	F	Ctrl	Arena 2	Trial 1	2_F
2	1	119447	279_C_2	6/23/2022	10/4/2022	ContextAtraining	3	F	Ctrl	Arena 3	Trial 1	3_F
3	1	119447	279_C_2	6/23/2022	10/4/2022	ContextAtraining	4	F	Ctrl	Arena 4	Trial 1	4_F
4	1	119476	279_C_2	6/23/2022	10/4/2022	ContextAtraining	5	F	Ctrl	Arena 1	Trial 2	5_F
...	...	...	...	...	...	...	...	...	...	...	...	...
91	1	119470	237_E_3	6/26/2022	11/3/2022	ContextBTest	7	M	ELS	Arena 3	Trial 37	7_M
92	1	119470	237_E_3	6/26/2022	11/3/2022	ContextBTest	8	M	ELS	Arena 4	Trial 38	8_M
93	1	113342	265_E_5	6/28/2022	11/3/2022	ContextBTest	9	M	ELS	Arena 1	Trial 38	9_M
94	1	113342	265_E_5	6/28/2022	11/3/2022	ContextBTest	10	M	ELS	Arena 2	Trial 39	10_M
95	1	113342	265_E_5	6/28/2022	11/3/2022	ContextBTest	11	M	ELS	Arena 3	Trial 39	11_M

96 rows × 12 columns

Merge freezing data with metadata

There are also arenas here that do not correspond with metadata and therefore contain NaN in this merged table. Remove using `dropna` again.

In [21]:
```
sefl_df = etho_tidy.merge(etho_meta_mod, how='outer', on= ['Experiment','Trial', 'Arena']) #, indicator = True)
sefl_df = sefl_df.dropna(axis = 'rows')
sefl_df
```

Out[21]:

	Trial	Experiment	Time_Bin	Arena	freezing_duration(s)	Bout(s)	PcentFreezing	Time(s)	Cohort	Cage	Dam ID	DOB	Exp Start	Mouse	Sex	Conditic
0	Trial 1	ContextAtraining	0:00:00-0:00:30	Arena 1	0.16	30.0	0.533333	30.0	1.0	119447.0	279_C_2	6/23/2022	10/4/2022	1.0	F	Ct
1	Trial 1	ContextAtraining	0:00:30-0:01:00	Arena 1	0.64	30.0	2.133333	60.0	1.0	119447.0	279_C_2	6/23/2022	10/4/2022	1.0	F	Ct
2	Trial 1	ContextAtraining	0:01:00-0:01:30	Arena 1	1.80	30.0	6.000000	90.0	1.0	119447.0	279_C_2	6/23/2022	10/4/2022	1.0	F	Ct
3	Trial 1	ContextAtraining	0:01:30-0:02:00	Arena 1	5.68	30.0	18.933333	120.0	1.0	119447.0	279_C_2	6/23/2022	10/4/2022	1.0	F	Ct
4	Trial 1	ContextAtraining	0:02:00-0:02:30	Arena 1	2.40	30.0	8.000000	150.0	1.0	119447.0	279_C_2	6/23/2022	10/4/2022	1.0	F	Ct
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
1120	Trial 13	ContextATest	0:03:00-0:03:30	Arena 4	30.00	30.0	100.000000	210.0	1.0	119470.0	237_E_3	6/26/2022	10/5/2022	8.0	M	El
1121	Trial 13	ContextATest	0:03:30-0:04:00	Arena 4	29.76	30.0	99.200000	240.0	1.0	119470.0	237_E_3	6/26/2022	10/5/2022	8.0	M	El
1122	Trial 13	ContextATest	0:04:00-0:04:30	Arena 4	29.40	30.0	98.000000	270.0	1.0	119470.0	237_E_3	6/26/2022	10/5/2022	8.0	M	El
1123	Trial 13	ContextATest	0:04:30-0:05:00	Arena 4	27.88	30.0	92.933333	300.0	1.0	119470.0	237_E_3	6/26/2022	10/5/2022	8.0	M	El
1124	Trial 13	ContextATest	0:05:00-0:05:30	Arena 4	0.04	30.0	0.133333	330.0	1.0	119470.0	237_E_3	6/26/2022	10/5/2022	8.0	M	El

848 rows × 17 columns

Analysis and Figures

Check column names for group analysis and data filtering

In [22]:
```
sefl_df.columns
```

Out[22]:
```
Index(['Trial', 'Experiment', 'Time_Bin', 'Arena', 'freezing_duration(s)',
       'Bout(s)', 'PcentFreezing', 'Time(s)', 'Cohort', 'Cage', 'Dam ID',
       'DOB', 'Exp Start', 'Mouse ', 'Sex', 'Condition', 'Mouse ID'],
      dtype='object')
```

Isolate each day of SEFL Training from the `Experiment` column into separate data frames

In [23]:
```
context_A_train = sefl_df[(sefl_df.Experiment == 'ContextAtraining')]
context_A_test = sefl_df[(sefl_df.Experiment == 'ContextATest')]
context_B_test = sefl_df[(sefl_df.Experiment == 'ContextBTest')]
```

We are interested in the effect of SEFL on animals that have experienced early life stress. Initially, we will examine group differences independently of sex, and later explore the sex differences.

We subset based on the relevant group and individual information, `Mouse ID`, `Sex`, `Condition`, and then calculate the mean of `PcentFreezing` for each time bin and `Condition`.

In [24]:
```
A_train_subset = context_A_train[['Mouse ID','Sex','Condition','Time(s)', 'PcentFreezing']]
A_train_els= A_train_subset.groupby(['Condition','Time(s)']).mean()

A_test_subset = context_A_test[['Mouse ID','Sex','Condition','Time(s)', 'PcentFreezing']]
A_test_els= A_test_subset.groupby(['Condition','Time(s)']).mean()

B_test_subset = context_B_test[['Mouse ID','Sex','Condition','Time(s)', 'PcentFreezing']]
B_test_els= B_test_subset.groupby(['Condition','Time(s)']).mean()
```

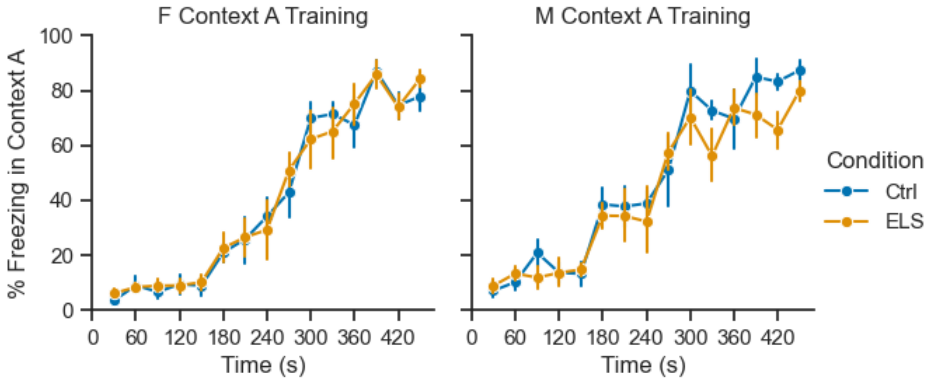
In [25]:
```
#ignore this for now, it just makes things look good
sns.set_style("ticks")
sns.set_context("notebook")
```

We will now generate faceted graphs of the freezing data for each context, separating Males and Females.

In [26]:
```
g = sns.FacetGrid(data = A_train_subset,col= 'Sex', hue = 'Condition',palette = 'colorblind')
g.map_dataframe(sns.lineplot,
                x = 'Time(s)',
                y = 'PcentFreezing',
                estimator='mean',
                ci = 68, #for normally distributed data, a 68% CI will correspond to SEM
                marker = 'o',
                err_style = 'bars')
g.set_axis_labels('Time (s)', '% Freezing in Context A')
g.set_titles(col_template="{col_name} Context A Training", row_template="{row_name}")
```

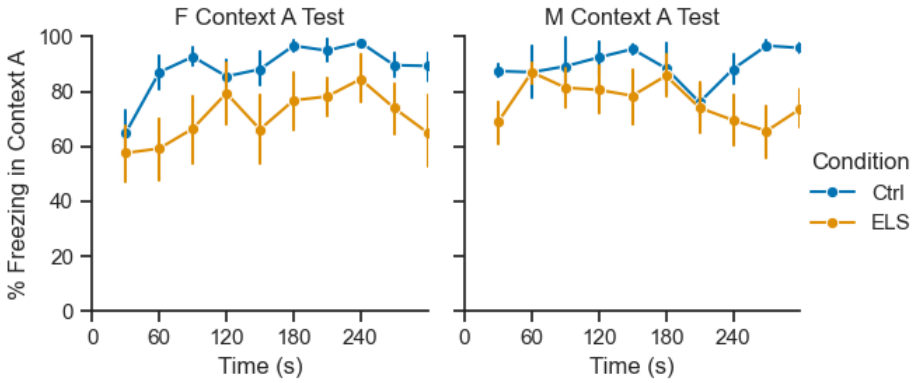
```
g.set(ylim=(0, 100), xlim=(0,470), xticks= np.arange(0, 470, 60))
g.add_legend()
```

Out[26]:
```
<seaborn.axisgrid.FacetGrid at 0x1bfe8718190>
```



In [27]:
```
g2 = sns.FacetGrid(data = A_test_subset,col= 'Sex', hue = 'Condition',palette = 'colorblind')
g2.map_dataframe(sns.lineplot,
                 x = 'Time(s)',
                 y = 'PcentFreezing',
                 estimator='mean',
                 ci = 68, #for normally distributed data, a 68% CI will correspond to SEM
                 marker = 'o',
                 err_style = 'bars')
g2.set_axis_labels('Time (s)', '% Freezing in Context A')
g2.set_titles(col_template="{col_name} Context A Test", row_template="{row_name}")
g2.set(ylim=(0, 100), xlim=(0,300), xticks= np.arange(0, 300, 60))
g2.add_legend()
```

Out[27]:
```
<seaborn.axisgrid.FacetGrid at 0x1bfe86f6bf0>
```



In [28]:
```
g3 = sns.FacetGrid(data = B_test_subset,col= 'Sex', hue = 'Condition',palette = 'colorblind')
g3.map_dataframe(sns.lineplot,
                 x = 'Time(s)',
                 y = 'PcentFreezing',
                 estimator='mean',
                 ci = 68, #for normally distributed data, a 68% CI will correspond to SEM
                 marker = 'o',
                 err_style = 'bars')
g3.set_axis_labels('Time (s)', '% Freezing in Context B')
g3.set_titles(col_template="{col_name} Context B Test", row_template="{row_name}")
g3.set(ylim=(0, 100), xlim=(0,270), xticks= np.arange(0, 270, 60))
g3.add_legend()
```

Out[28]:
```
<seaborn.axisgrid.FacetGrid at 0x1bfe9089c00>
```