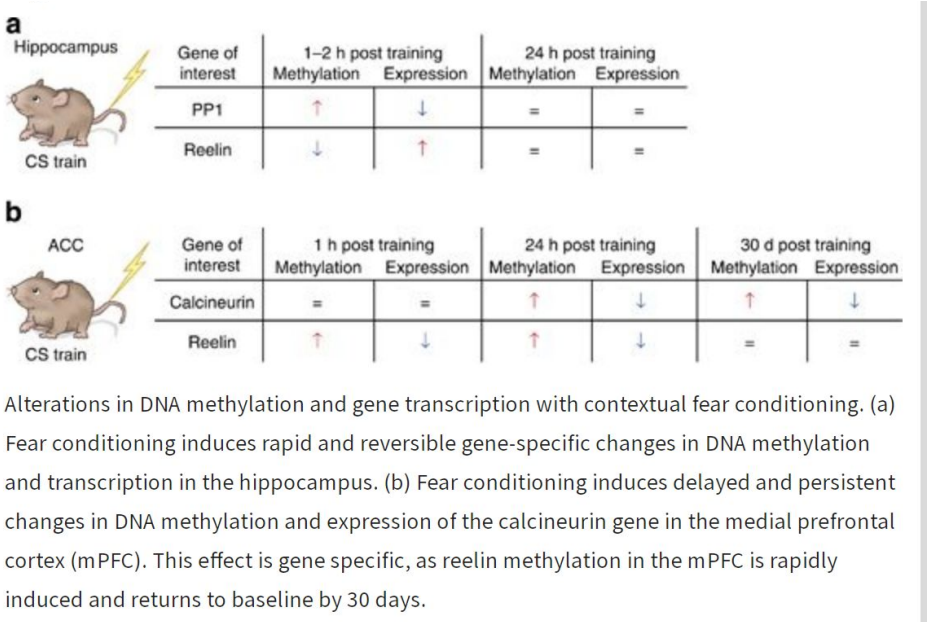
Figure 1a



a

Hippocampus

CS train

Gene of interest	1–2 h post training		24 h post training	
	Methylation	Expression	Methylation	Expression
PP1	↑	↓	=	=
Reelin	↓	↑	=	=

b

ACC

CS train

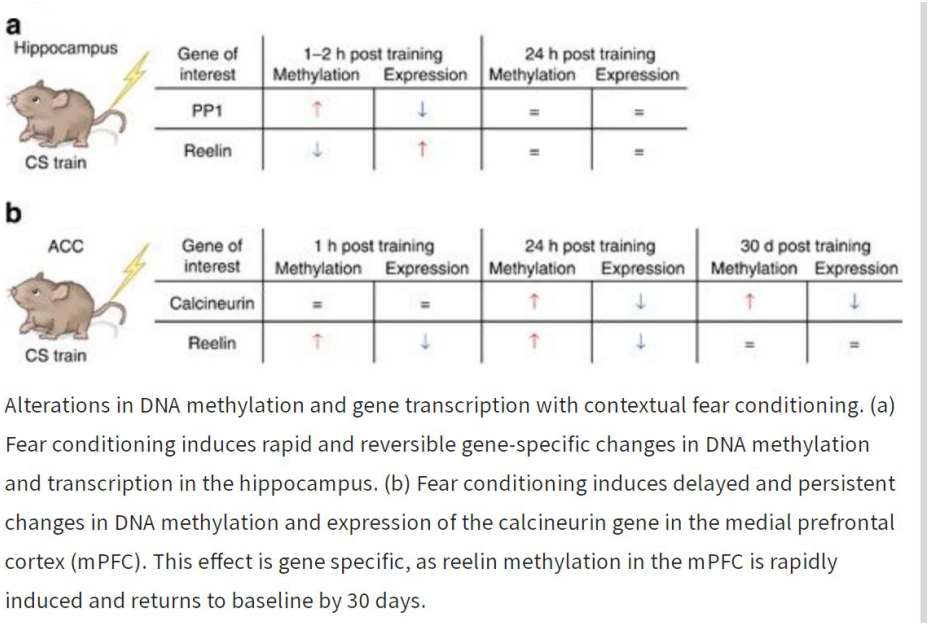
Gene of interest	1 h post training		24 h post training		30 d post training	
	Methylation	Expression	Methylation	Expression	Methylation	Expression
Calcineurin	=	=	↑	↓	↑	↓
Reelin	↑	↓	↑	↓	=	=

Alterations in DNA methylation and gene transcription with contextual fear conditioning. (a) Fear conditioning induces rapid and reversible gene-specific changes in DNA methylation and transcription in the hippocampus. (b) Fear conditioning induces delayed and persistent changes in DNA methylation and expression of the calcineurin gene in the medial prefrontal cortex (mPFC). This effect is gene specific, as reelin methylation in the mPFC is rapidly induced and returns to baseline by 30 days.

Question:
In the Hippocampus, what effect did the fear conditioning have on general expression in the long term? Why do you think this was the case, and what might the hippocampus do that correlates with this expression change?

Zovkic, I. B., Sweatt, J. D. Epigenetic Mechanisms in Learned Fear: Implications for PTSD. *Neuropsychopharmacology* **38**, 77-93 (2013).

Figure 1b



a

Hippocampus

CS train

Gene of interest	1–2 h post training		24 h post training	
	Methylation	Expression	Methylation	Expression
PP1	↑	↓	=	=
Reelin	↓	↑	=	=

b

ACC

CS train

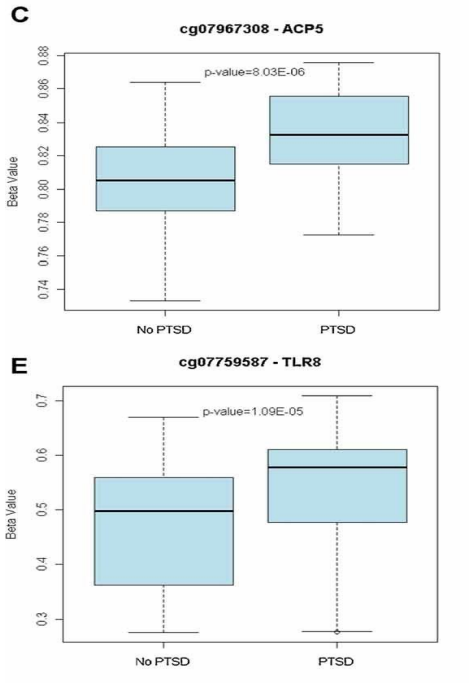
Gene of interest	1 h post training		24 h post training		30 d post training	
	Methylation	Expression	Methylation	Expression	Methylation	Expression
Calcineurin	=	=	↑	↓	↑	↓
Reelin	↑	↓	↑	↓	=	=

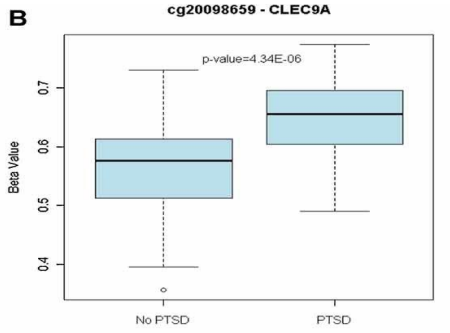
Alterations in DNA methylation and gene transcription with contextual fear conditioning. (a) Fear conditioning induces rapid and reversible gene-specific changes in DNA methylation and transcription in the hippocampus. (b) Fear conditioning induces delayed and persistent changes in DNA methylation and expression of the calcineurin gene in the medial prefrontal cortex (mPFC). This effect is gene specific, as reelin methylation in the mPFC is rapidly induced and returns to baseline by 30 days.

Question:
In part b, what changes in long-term gene expression do you notice, if any? Why are these changes not uniform as in part a, and what role do you think these genes have that would necessitate this difference?

Zovkic, I. B., Sweatt, J. D. Epigenetic Mechanisms in Learned Fear: Implications for PTSD. *Neuropsychopharmacology* **38**, 77-93 (2013).

Figure 2a



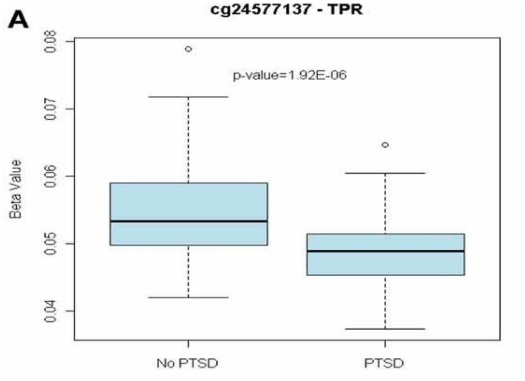


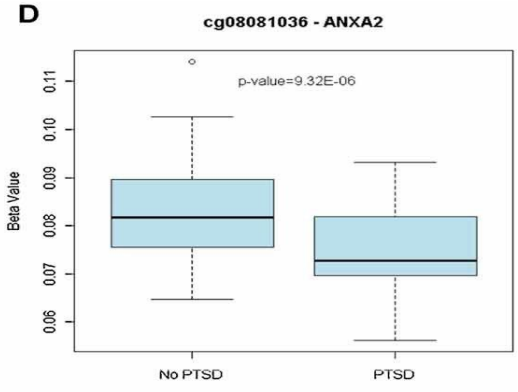
Question:
What is the difference in DNA Methylation between these three genes in individuals with or without PTSD? What role(s) do you think these genes have that would correlate them with PTSD?

CpG sites in *TPR*, *CLEC9A*, *ACP5*, *ANXA2*, and *TLR8* are differentially methylated in subjects with PTSD, and *NPFFR2* methylation is associated with Total Life Stress. The methylation level (β-value, box plot of average methylation value) for each subject is plotted on the vertical axis while PTSD diagnosis is plotted on the horizontal axis. Box plots of methylation values in PTSD cases (N = 50) and controls (N = 60) are shown for TPR (**A**), CLEC9A (**B**), ACP5 (**C**), ANXA2 (**D**), and TLR8 (**E**). **F**: Total life stress (TLS) scores for all subjects (N = 110) are plotted on the horizontal axis and NPFFR2 methylation β values are presented as a scatter plot on the vertical axis. All six CpG sites met experiment-wide criteria for significance (FDR < 0.05).

Smith A. K., *et al*. Differential Immune System DNA Methylation and Cytokine Regulation in Post-Traumatic Stress Disorder. *American Journal of Medical Genetics Part B* **156**, 700–708 (2011).

Figure 2b





Question:
What is the difference in DNA Methylation between these two genes in individuals with or without PTSD? What role(s) do you think these genes have that would correlate them with PTSD?

CpG sites in *TPR*, *CLEC9A*, *ACP5*, *ANXA2*, and *TLR8* are differentially methylated in subjects with PTSD, and *NPFFR2* methylation is associated with Total Life Stress. The methylation level (β-value, box plot of average methylation value) for each subject is plotted on the vertical axis while PTSD diagnosis is plotted on the horizontal axis. Box plots of methylation values in PTSD cases (N = 50) and controls (N = 60) are shown for TPR (**A**), CLEC9A (**B**), ACP5 (**C**), ANXA2 (**D**), and TLR8 (**E**). **F**: Total life stress (TLS) scores for all subjects (N = 110) are plotted on the horizontal axis and NPFFR2 methylation β values are presented as a scatter plot on the vertical axis. All six CpG sites met experiment-wide criteria for significance (FDR < 0.05).

Smith A. K., *et al*. Differential Immune System DNA Methylation and Cytokine Regulation in Post-Traumatic Stress Disorder. *American Journal of Medical Genetics Part B* **156**, 700–708 (2011).

Figure 3a

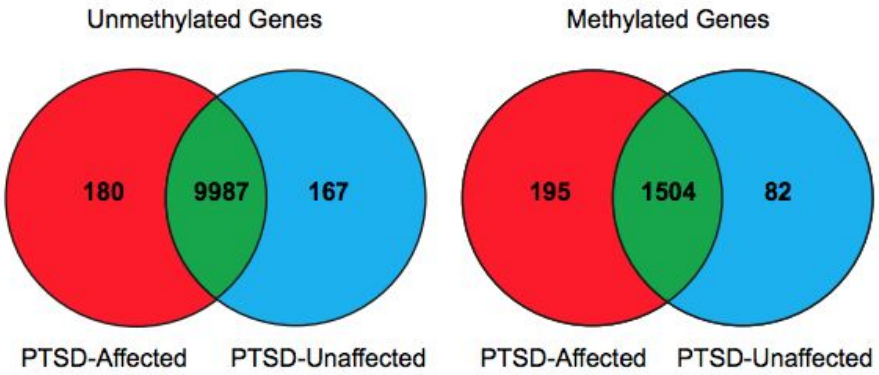


Fig. 1. Number of methylated and umethylated genes according to PTSD status. Red indicates the genes uniquely methylated or unmethylated in the PTSD-affected group, blue indicates the genes uniquely methylated or unmethylated in the PTSD-unaffected group, and green indicates the genes commonly methylated or unmethylated in both groups. Methylated genes were defined as those genes with average β values of >0.8, and unmethylated genes were defined as those genes with average β values of <0.2.

Question:

What types of genes do you think PTSD-Affected individuals have uniquely methylated? PTSD-Unaffected individuals?

Uddin, M., *et al.* Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proceedings of the National Academy of Sciences* 107 (20) 9470-9475; (2010).

Figure 3b

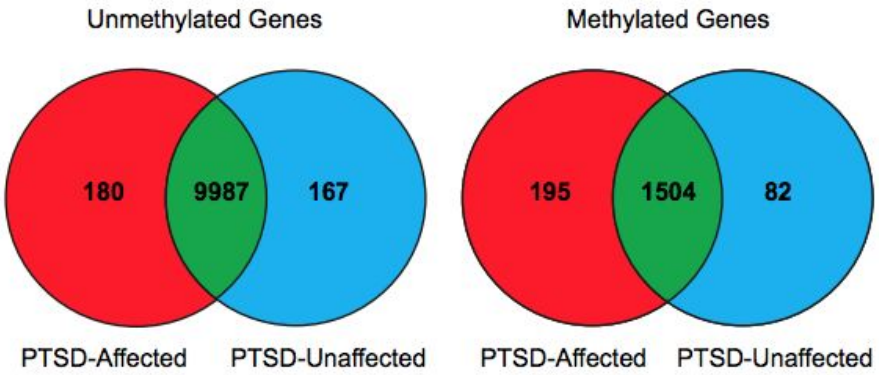


Fig. 1. Number of methylated and umethylated genes according to PTSD status. Red indicates the genes uniquely methylated or unmethylated in the PTSD-affected group, blue indicates the genes uniquely methylated or unmethylated in the PTSD-unaffected group, and green indicates the genes commonly methylated or unmethylated in both groups. Methylated genes were defined as those genes with average β values of >0.8, and unmethylated genes were defined as those genes with average β values of <0.2.

Question:

What types of genes do you think PTSD-Affected individuals have uniquely unmethylated? PTSD-Unaffected individuals?

Uddin, M., *et al.* Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proceedings of the National Academy of Sciences* 107 (20) 9470-9475; (2010).

Figure 4

Table 1: Comparison of CpG sites mapping to reported genes in PTSD and MDD literatures

From: An epigenome-wide DNA methylation study of PTSD and depression in World Trade Center responders

Gene	PTSD			MDD			
	N	Hypo	Hyper	N	Hypo	Hyper	Reference
FKBP5	1	1	0	1	1	0	Binder *et al.*[12] Klengel *et al.*[13] Weder *et al.*[32]
NR3C1	1	1	0	6	6	0	McGowan *et al.*[10] Yehuda *et al.*[11] Weder *et al.*[32]
BDNF	4	3	1	2	2	0	Roth *et al.*[16] Weder *et al.*[32]
SLC6A4	0	0	0	1	1	0	Chang *et al.*[14] Koenen *et al.*[15]
TPR	0	0	0	0	0	0	Smith *et al.*[26]
CLEC9A	0	0	0	0	0	0	Smith *et al.*[26]
ANAPC5	0	0	0	0	0	0	Smith *et al.*[26]
ANXA2	1	1	0	2	2	0	Smith *et al.*[26]
TLR8	1	0	1	0	0	0	Smith *et al.*[26]
GRIK2	0	0	0	2	2	0	Nagy *et al.*[31]
BEGAIN	0	0	0	0	0	0	Nagy *et al.*[31]
GRIN1	0	0	0	2	0	2	Weder *et al.*[32]
ID3	0	0	0	0	0	0	Weder *et al.*[32]
TPPP	0	0	0	2	0	2	Weder *et al.*[32]
ADCYAP1	3	2	1	2	2	0	Ressler *et al.*[17]

Abbreviations: Hyper, hypermethylation in case relative to control; Hypo, hypomethylation in case relative to control; MDD, major depressive disorder; PTSD, posttraumatic stress disorder. *N*, number of CpG sites at nominal *P*□0.05.

Question:
What relationship(s) do you think these genes have between MDD and PTSD?
Can we confirm that the presence (or absence) of DNA Methylation within these genes is to blame for these disorders based on the table alone?
Why or why not?

Kuan, P-F, *et al*. An epigenome-wide DNA methylation study of PTSD and depression in World Trade Center responders. *Translational Psychiatry* **7**, 1-8 (2017).