Supplementary Methods

Fear conditioning and extinction measurements and task: In this study a classical fear conditioning paradigm was used. Briefly, this paradigm consisted of a habituation, conditioning and extinction phase. The conditioned stimuli (CS) consisted of yellow and blue squares and the unconditioned stimulus (US) was an aversive electrical stimulation to the participant's wrist. Before the imaging session, MR compatible carbon electrodes (Kendall H135TSG) for electrical stimulation were placed on the right wrist 1-2 cm apart. All subjects selected a level of shock intensity that was experienced as highly annoying but not painful, to be used in the experiment. Intensities could vary between 1 and 99 mA with a constant voltages of 400 V.

In the habituation phase, the 4 CS+ and 4 CS- were presented in a pseudo-random manner. During the conditioning phase, the CS+ was paired with the US at a partial reinforcement rate of 33%. There were 18 CS- trials, 12 CS+ trials that were unpaired with the US and 6 CS+ trials that were paired with the US. The US directly followed the offset of the CS+. After a break of approximate 30 seconds the extinction phase began. During this phase 18 CS+ trials and 18CS- trials were presented, but none of the CS+ trials were paired with an electrical shock. For each trial during the experiment, the CS+ and CS- were presented for 4 seconds, the US was presented for 2 ms, and the intertrial interval varied between 6.5 and 9.5 seconds during which a fixation cross was presented. Before onset of each phase subjects were instructed that they could receive electrical shocks and that they should pay attention on the relation between the visual stimuli presented and the electrical shocks.

FMRI data acquisition and first-level analysis: Images were acquired on a 3.0-T Achieva full-body scanner (Philips Medical Systems, Best, the Netherlands) using a 32 channel SENSE head coil. Echo planar images (EPIs) were taken covering the whole brain, with a total of 37 ascending axial slices (3x3x3mm voxel size; slice gap 3mm; TR/TE 2000ms/28ms; matrix 80x80). Also a T1-3D high resolution anatomical scan (TR/TE 8.2/3.7; matrix 240x187; 1x1x1 voxel; transverse slices) was taken.

fMRI data were analyzed using SPM8. Preprocessing included realignment, slice-time correction, coregistration of the structural and functional scans, normalization to MNI-space based on the segmented structural scan and smoothing with a kernel of 8 mm full-width at half maximum. First level models included separate regressors for CS-, CS+ paired with the US, CS+ unpaired with the US and the US itself, during habituation, conditioning and extinction blocks. These regressors were convolved with the canonical hemodynamic response function. Six realignment parameters were included as regressors of no interest. A high pass filter (1/128 Hz) was included in the first level model to correct for low frequency signal drift.

Physiological data acquisition and analysis: Skin conductance was measured simultaneously with fMRI acquisition. Skin-conductance response (SCRs) was measured using an MRI compatible GSR set with Ag/AgCl electrodes covered in isotonic gel (Brain Products GmbH, Germany) with a constant voltage of 0.5V. Electrodes were placed at the medial phalanges of the index and middle finger. SCR was recorded using Net Station (version 4.5.2) at a sample rate of 250 Hz. SCR was recorded from the onset to the end of the fear conditioning paradigm. The MRI artefact was removed using the MRI artifact removal tool in NetStation. Subsequently, the SCR signal was low-pass filtered using a cut-off value of 2 Hz.

Exploring the effects of poly-substance abuse on the neural correlates of aversive conditioning and extinction learning.
The relation between state anxiety and days since last use was tested by adding these covariates to the fMRI model, for early conditioning, late conditioning, early extinction and late extinction. To explore whether abnormalities in aversive conditioning and extinction were related to the amount of substance used, we aimed to test the relation between cannabis, nicotine, alcohol and cocaine use and the neural correlates of aversive conditioning and extinction learning. The data of drug use could not be transformed to have a normal distribution. Therefore cocaine users were divided into mild or severe users of alcohol, nicotine and cocaine, based on the median split mild alcohol use < 19.9 glasses a week, severe alcohol use ≥ 19.9 glasses a week; no or mild nicotine use < FTND score of 5, severe nicotine use ≥ FTND score of 5; mild cocaine use <7.6 grams per month, severe cocaine use ≥ 7.6 grams per month. Cannabis use was based on whether or not they used less than weekly (no to mild use ranging from 0 to 3.8 days per month) or more than weekly (severe user ranging from 4.3 to 30.3 days per month). Chi-square tests indicated that there was no relation between mild or severe use of the four substances.

Supplementary Results

Main task effects
Table S1 and Figure S1 show the main task effect of aversive conditioning and extinction learning on brain activation.

The correlation between days since last use and fMRI during fear conditioning and extinction
The regression analysis did not show any significant relation between days since last use and any fMRI measure.

The correlation between SCR and fMRI during fear conditioning and extinction
Table S2 and Figure S2 show the brain regions of which the activation is significantly correlated to the skin conductance response, during aversive conditioning and extinction learning.

Effect state anxiety and poly-substance use on neural correlates of fear extinction
Table S3 and Figure S3 show the correlation between state anxiety and aversive conditioning and extinction learning as well as the difference between mild and heavy cannabis and cocaine users.

FIGURE S1–S3. Figure S1 (left-hand panel) shows the main effects of stimulus type, for all four phases of the task. In red the CS+>CS- contrast, in blue the CS->CS+ contrast. Figure S2 (center panel) shows the correlations between SCRs and fMRI response, during all four phases of the task. Red shows the positive correlations, and blue shows the negative correlations. Figure S3 (right-hand panel) shows the effects of state anxiety, cannabis and cocaine use.

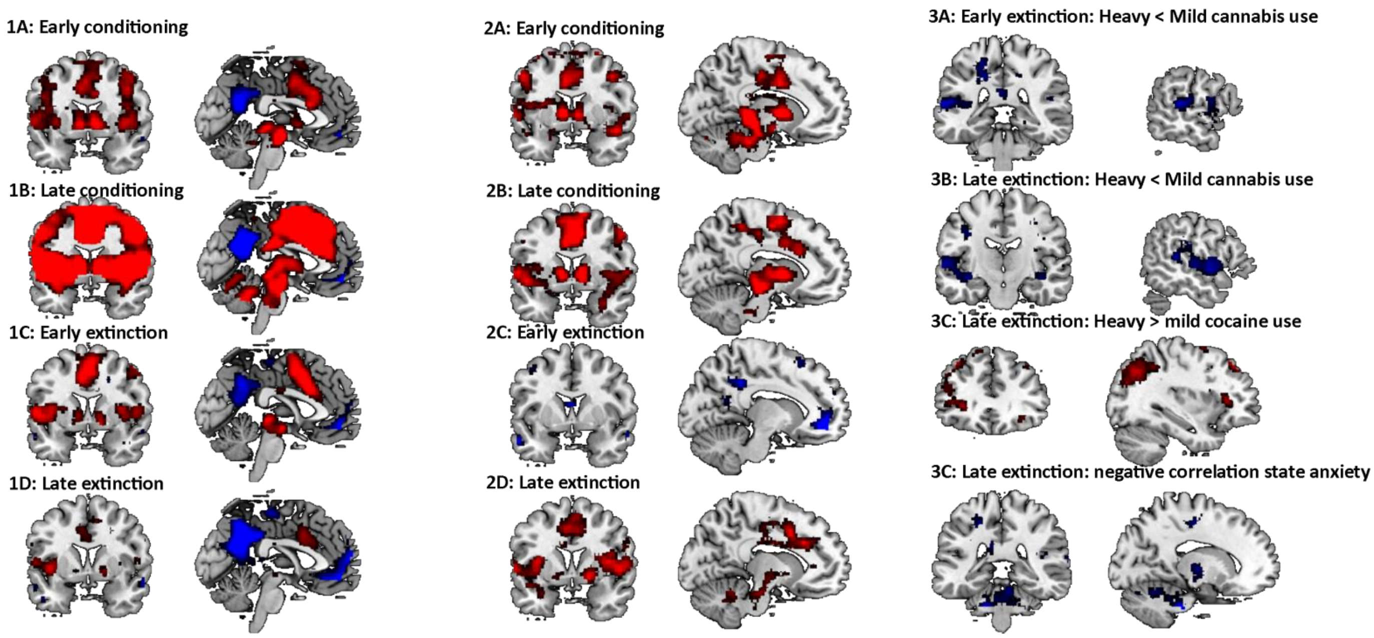

TABLE S1. Neural correlates of aversive conditioning and extinction: main task effects

	Cluster size *# voxels*	Cluster *P-value*	Peak-Voxel *z-value*	Voxel *MNI-coordinates*			Voxel region
Early Conditioning							
CS+ > CS-	4703	<0.001	5.79	32	24	-4	R Insula
			5.18	48	18	4	R Inferior frontal gyrus
			3.92	42	4	38	R Precentral gyrus
			3.8	44	8	14	R Rolandic operculum
	3831	<0.001	4.31	-10	2	0	L Pallidum
			3.03	-8	-8	10	L Thalamus
			3.19	18	-10	4	R Thalamus
	3420	<0.001	5.34	-30	22	-2	L Insula
			4.2	-56	6	2	L Inferior frontal gyrus
			3.47	-44	-4	54	L Precentral gyrus
	966	0.028	4.85	60	-40	22	R Superior temporal gyrus
	1111	0.015	4.56	-56	-26	22	L Supramarginal gyrus
			2.42	-62	-42	16	L Superior temporal gyrus
	3697	<0.001	4.38	0	16	22	L Anterior cingulate gyrus
			4.24	-4	0	44	L Middle cingulate
			4.35	10	14	36	R Middle cingulate
			3.69	6	14	56	R Superior motor area
CS- > CS+	1802	0.001	4.49	-2	-58	26	L Precuneus
			3.62	10	-48	14	R Precuneus
			3.57	-6	-42	34	L Posterior cingulate gyrus
Late conditioning							
CS+ > CS-	59758	<0.001	Inf	-30	24	4	L Insula
			Inf	36	28	-2	R Insula
			Inf	-6	8	38	L Middle cingulate gyrus
			Inf	-2	2	52	L Superior motor area
			Inf	2	4	52	R Superior motor area
			Inf	-60	-30	22	L Superior temporal gyrus
			Inf	64	-40	34	R Supramarginal gyrus
			Inf	-48	2	0	L Rolandic operculum
	57	0.001	4.01	-20	0	-12	L Amygdala[a]
	16	0.045	2.94	32	-2	-12	R Amygdala[a]
CS- > CS+	2850	<0.001	6.1	-4	-60	24	L Precuneus
			4.03	12	-52	12	R Precuneus
			3.94	14	-48	10	R Calcarine cortex
	1235	0.008	5.96	-46	-68	28	L Angular gyrus
			2.46	-22	-86	32	L Superior occipital gyrus
			2.64	-28	-84	34	L Middle occipital gyrus
	982	0.026	5.83	46	-60	26	R Angular gyrus
			3.18	32	-80	40	R Superior occipital gyrus
			3.12	38	-76	38	R Middle occipital gyrus
	962	0.029	4.38	-4	48	-12	L Medial orbital frontal gyrus
			4.35	2	36	-18	R Gyrus rectus
			4.26	-12	58	10	L Superior frontal gyrus
			3.27	14	58	8	R Superior frontal gyrus
			3.31	-26	28	50	L Middle frontal gyrus
			3.15	8	38	-12	R Medial orbital frontal gyrus
Early extinction							
CS+ > CS-	1092	0.01	5.77	-52	-30	24	L Supramarginal gyrus
	3799	<0.001	5.56	-36	14	4	L Insula
			4.35	-52	4	6	L Inferior frontal gyrus
			3.8	-42	0	12	L Superior temporal gyrus
			3.47	-10	2	2	L pallidum
			3.79	14	2	-2	R Pallidum
			3.62	-24	4	8	L Putamen
			3.53	8	-20	-8	L Red Nucleus
			2.57	-10	-16	0	L Thalamus
	2319	<0.001	5.47	34	24	-4	R Insula
			4.68	42	22	6	R Inferior frontal gyrus
			3.59	52	6	4	R Rolandic opperculum
			2.57	42	10	-26	R Superior temporal pole
	3211	<0.001	4.84	-8	10	42	L Middle cingulate gyrus
			4.64	4	16	32	R Middle cingulate gyrus
			4.57	-4	10	44	L Superior motor area
			4.59	6	8	46	R Superior motor area
			4.57	-2	16	30	L Anterior cingulate gyrus

			3.24	4	28	18	R Anterior cingulate gyrus
			3.22	18	6	68	R Superior frontal gyrus
	942	0.021	4.3	64	-38	22	R Superior temporal gyrus
			4.29	60	-38	24	R Supramarginal gyrus
CS-> CS+	2562	<0.001	5.48	54	-62	24	R Angular gyrus
			4.33	36	-36	-14	R Parahippocampal gyrus
			3.99	26	-32	-16	R Fusiform gyrus
			3.91	46	-80	12	R Middle occipital gyrus
			3.91	48	-76	10	R Middle temporal gyrus
			3.61	46	-54	-16	R Inferior temporal gyrus
			2.85	12	-46	2	R Lingual gyrus
			2.82	14	-84	34	R Cuneus cortex
	4644	<0.001	4.85	-24	20	50	L Middle frontal gyrus
			5.35	-34	16	48	R Middle frontal gyrus
			5.04	-12	50	36	L Superior frontal gyrus
			4.82	-22	22	44	L Superior motor area
			4.46	-8	48	-12	L Medial Orbital frontal gyrus
	6673	<0.001	4.89	-60	-6	-16	L Middle temporal gyrus
			4.7	-44	-64	36	L Angular gyrus
			3.98	-30	-76	42	L Inferior parietal gyrus
			3.97	-6	-54	24	L Precuneus cortex
			3.86	-10	-50	26	L Posterior cingulate cortex
			3.79	-12	-48	36	L Middle cingulate cortex
			3.75	-22	-42	-12	L Fusiform gyrus
	1010	0.015	3.24	62	-4	26	R Postcentral gyrus
			3.14	34	-22	50	R Precentral gyrus
Late extinction							
CS+ > CS-	1884	<0.001	5.24	38	24	6	R Insula
			3.41	56	12	4	R Inferior frontal gyrus
			2.83	52	4	10	R Rolandic operculum
	1262	0.004	4.73	-32	18	8	L Insula
			3.21	-54	4	6	L Rolandic operculum
	1139	0.008	3.37	-4	10	36	L Middle cingulate gyrus
			3.7	12	16	40	R Middle cingulate gyrus
			3.2	8	20	28	R Anterior cingulate gyrus
			2.96	6	8	50	R Superior motor area
CS- > CS+	3930	<0.001	5.08	8	-22	56	R Superior motor area
			4.08	-10	-26	60	L Paracentral lobule
			4.85	10	-26	58	R Paracentral lobule
			4.56	58	-8	32	R Postcentral gryrus
			3.84	40	-24	54	R Precentral gyrus
	13937	<0.001	5.34	-14	-52	12	L Calcarine cortex
			5.34	-26	-20	-16	L Hippocampus
			5.14	26	-18	-18	R Hippocampus
			5.33	-8	-56	18	L Precunues
			5.11	-42	-70	38	L Angular gyrus
			5.06	-4	-54	28	L Posterior cingulate cortex
			4.65	22	-32	-10	R Parahippocampal gyrus
	4405	<0.001	5.01	-20	28	54	L Superior frontal gyrus
			4.76	4	40	-14	R Medial orbital frontal gyrus
			4.21	-24	20	50	L Middle frontal gyrus
	1105	0.009	4.83	-60	-10	36	L Postcentral gyrus
	974	0.018	4.49	26	32	46	R Middle frontal gyrus
			4.01	28	22	50	R Superior frontal gyrus
	1125	0.008	4.13	56	-64	20	R Middle temporal gyrus
			3.82	52	-62	24	R Angular gyrus
			3.68	44	-58	24	R Precuneus
			3.57	18	-84	38	R Cuneus
			2.82	42	-76	18	R Middle occipital gyrus
	71	0.003	3.80	-20	-8	-16	L Amygdala[a]

All results were $p<0.05$, cluster level family-wise error rate corrected with an initial height threshold of $p=0.01$ uncorrected
[a]Corrected for the volume of the right amygdala, $p_{peak\ voxel}<0.05$

TABLE S2. Neural correlates of aversive conditioning and extinction: interactions with skin conductance response

	Cluster size	Cluster	Voxel	Peak voxel			Voxel region
	# voxels	*P-value*	*z-value*	*MNI-coordinates*			
Early Conditioning (CS+>CS-)							
Positive correlations	13493	<0.001	5.4	30	-52	-28	R Cerebellum
			4.34	0	-14	6	L Thalamus
			4.13	46	6	-14	R Superior temporal pole
	4875	<0.001	4.53	-2	-6	66	L Superior motor area
			3.64	6	12	46	R Superior motor area
			4.49	-6	0	38	L Middle cingulate gyrus
			4.16	0	20	24	L Anterior cingulate gyrus
			3.99	-32	-4	64	L Precentral gyrus
			3.57	4	28	46	R Superior frontal gyrus
	893	0.017	3.93	56	-40	22	R Superior temporal gyrus
			3.45	52	-56	4	R Middle temporal cortex
			3.11	60	-32	26	R Supramarginal gyrus
			2.79	60	-22	48	R Postcentral gyrus
	763	0.036	3.8	-64	-34	32	L Supramarginal gyrus
			3.42	-62	-28	16	L Superior temporal gyrus
			3.08	-62	-18	22	L Postcentral gyrus
			2.87	-46	-20	16	L Rolandic operculum
	97	0.004	3.74	-18	-2	-12	L Amygdala
	28	0.020	3.26	18	-2	-14	R Amygdala
Late conditioning (CS+ > CS -)							
Positive correlations	6643	<0.001	4.98	2	8	42	R Middle cingulate gyrus
			3.68	-8	20	26	L Anterior cingulate gyrus
			4.36	8	20	28	R Anterior cingulate gyrus
			4.32	-10	-2	60	L Superior motor area
			3.92	10	6	64	R Superior motor area
			4.2	-60	-24	22	L Supramarginal gyrus
			4.01	-48	-22	22	L Rolandic operculum
			3.88	-34	-6	44	L Precentral gyrus
			3.68	-12	-38	52	L middle cingualte
	1896	0.001	4.67	60	10	4	R Rolandic operculum
			4.19	34	26	4	R Insula
			3.76	42	14	4	R Inferior frontal gyrus
			3.2	32	6	-24	R Superior temporal pole
			3.1	32	6	-32	R Parahippocampal gyrus
	6	0.025	3.12	34	4	-26	R Amygdala
Early extinction (CS+ > CS -)							
Negative correlation	2206	<0.001	4.78	-12	46	-6	L Medial orbital frontal gyrus
			3.79	16	48	-6	R Medial orbital frontal gyrus
			3.75	-18	54	24	L Superior frontal gyrus
			3.3	-34	32	-10	L Inferior frontal gyrus
			3.67	40	40	-8	R Inferior frontal gyrus
			3.61	-2	34	0	L Anterior cingulate gyrus
	1090	0.012	3.06	-8	-54	40	L Precuneus
			3.98	10	-52	24	R Precuneus
			3.86	-10	-40	36	L Middle cingulate gyrus
			2.89	8	-44	32	R Middle cingulate gyrus
			3.02	0	-52	32	L Posterior cingulate gyrus
			2.5	-6	-64	22	L Cuneus
			2.45	-12	-54	12	L Calcarine gyrus
	1037	0.015	3.79	58	-60	20	R Middle temporal gyrus
			3.6	42	-56	28	R Angular gyrus
			3.05	56	-58	38	R Inferior parietal gyrus
			2.93	36	-80	32	R Middle occipital gyrus
Late extinction (CS+ > CS -)							
Positive correlation	8709	<0.001	3.97	-38	-18	-4	L Insula
			4.61	52	8	2	R insula
			3.88	-38	-26	20	L Rolandic operculum
			4.14	42	6	12	R Rolandic operculum
			4.13	20	2	-4	R Pallidum
			3.95	-54	-22	18	L Supramarginal gyrus
	4187	<0.001	4.12	-8	8	42	L Middle cingulate gyrus
			3.98	10	16	36	R Middle cingulate gyrus
			3.94	-10	24	30	L Anterior cingulate gyrus

			3.82	6	14	50	R Superior motor area
	2047	<0.001	3.88	62	-34	20	R Superior temporal gyrus
			3.71	34	-14	-8	R Putamen
			3.59	48	-32	24	R Supramarginal gyrus
			2.5	42	-14	36	R Rrecentral gyrus

All results were $p<0.05$, cluster level family-wise error corrected and $p<0.01$ voxel level uncorrected

[a]Corrected for the volume of the right amygdala, $p<0.05$ voxel level family-wise error corrected

TABLE S3. Neural correlates of aversive conditioning and extinction: exploring the relation with state anxiety, and the type and amount of substance used

	Cluster size *# voxels*	Cluster *P-value*	Voxel *z-value*	Peak voxel *MNI-coordinates*			Voxel region
<u>Early extinction (CS+ > CS -)</u>							
Mild > heavy cannabis use	1173	0.012	3.51	-58	-34	12	L Superior temporal gyrus
			3.26	-60	-24	16	Left Supramarginal gyrus
			3,16	-62	-2	14	L Postcentral gyrus
			3,15	-50	-22	0	L Middle Temporal gyrus
			3,01	-38	-26	14	L Herschl Gyrus
			2,9	-56	0	10	L Rolandic Opperculum
<u>Late extinction (CS+ > CS -)</u>							
Negative correlation STAI	1912	0.001	4.08	12	-52	-16	L/R Cerebellum
Mild > Heavy cannabis use	1872	0.001	4,11	-60	6	8	Left inferior frontal gyrus
			3,57	-52	-4	-2	L Superior temporal gyrus
			3,38	-60	-34	8	Left middle temporal gyrus
			3,17	-50	-26	28	L Supramarginal gyrus
			3,72	-20	-72	38	L Superior occipital gyrus
Heavy > Mild cocaine use	4464	<0.001	3,59	-34	-64	38	L Middle occipital gyrus
			3,57	-28	-74	42	L inferior parietal
			3,5	-26	-74	46	L Superior parietal gyrus
			3,5	10	-34	36	R middle cingulate gyrus
			3,39	-26	-70	36	L Superior occipital gyrus
			3,3	-8	-46	30	L Posterior cingulate
			3,29	-10	-52	50	L Precuneus
	2104	<0.001	3,67	-40	28	8	L inferior frontal gyrus
			3,49	-34	34	44	L Middle frontal gyrus
			3,38	-32	26	10	L Insula
			3,34	-8	48	48	L Superior frontal gyrus
			3,05	-40	10	40	L Precentral gyrus

All results were $p<0.05$, cluster level family-wise error corrected and $p<0.01$ voxel level uncorrected