SUPPLEMENTARY MATERIALS

Title: Transitions in infant learning are modulated by dopamine within the amygdala

Authors:

Gordon A. Barr (barrg@email.chop.edu)

Stephanie Moriceau

Kiseko Shionoya

Kyle Muzny

Puhong Gao

Shaoning Wang

Regina M. Sullivan (regina.sullivan@nyumc.org)

Supplementary Information about the model

Maternal odor learning; facilitated approach learning & attenuated avoidance learning in young animals.

Maternal odors guide rat pups to their mother and control social interactions with her. Pups must learn to approach the odor, and rat pups of this age have developed unique learning capabilities, which are likely a mammalian parallel to imprinting, that permit this early attachment learning, *even for noxious stimuli*. For example, a novel odor paired with either the mother, milk, tactile stimulation (stroking to mimic grooming), tailpinch or 0.5mA shock is sufficient for pups to learn to later approach that odor. More importantly, this odor can now substitute for the maternal odor and support more complex social interactions with the mother[1, 2]. Why would noxious stimulation support attachment learning? We suggest this is related to the rough handling pups receive from the mother as part of the normal mother-infant interaction. Specifically, the mother entering and leaving the nest drags still-attached pups out of the nest and steps on others, producing what appears to be distress in the pup (Supplementary Table 1). Although pups respond immediately to noxious stimulation that appears to be painful[3-8], rough handling by the mother is sufficient to produce a subsequent approach to the odor[9].

This attenuation of odor-aversion conditioning during pups' early life is not the only restriction on learning for pups. Both inhibitory conditioning and passive avoidance are also attenuated [10,

[11]. Furthermore, learning paradigms that retard or inhibit learning in adults, such as latent inhibition (CS pre-exposure) and learned irrelevance (uncorrelated presentations of the CS and reward), actually enhance or have no effect on the young infant rat's learning[12-18]. Thus, rat pups have myriad unique learning capabilities that both potentiate the learning required for pups to maintain proximity to their mother and also prevent pups from avoiding the mother.

SUPPLEMENTARY TABLE 1. Pup and Maternal Interactions					
MATERNAL BEHAVIOR				**PUP BEHAVIOR**	
Steps on pups	*Rough pups retrieving*	*Grooming/ licking pups*	*Away from nest*	*Nursing*	*Ultrasonic and audible vocalizations*
2.25±0.2%	3.5±0.4%	36±2%	10.5±1.1%	80±4.3%	5.1±0.9%

Mother-litter interactions were observed for 90 minutes during the morning when pups were 7 to 9-days-old. As indicated by the percent number of occurrences of particular behaviors, most maternal behavior involved nurturing pups, although rough maternal behavior occurred and elicited vocalizations.

Limitations on aversive learning in infancy in other species.

Attenuation of avoidance/inhibitory learning is widespread across species and suggests a phylogenetically preserved neural system for attachment, a suggestion originally put forth by Bowlby[19]. This was first demonstrated in birds during imprinting: shocking the infant as it followed a surrogate caregiver enhances following of the surrogate. As the imprinting critical period ends, the same shock results in avoidance of the surrogate [20]. In similar work in young dogs [21], shock or maltreatment by a human caregiver, produces learned attachment to that caregiver. In nonhuman primates, the young of abusive mothers form and maintain strong attachments to their caregiver [22-26]. Clinically, an analogous situation in humans includes children who form and maintain strong attachments to their abusive caregivers [19, 27]. Thus the infant rat model showing preference learning for odors paired with normally noxious stimuli that can in turn produce approach responses and nipple attachment in procedures conducted within the nest and in outside the nest in more controlled learning studies [9, 28-31].

Development of fear learning and the amygdala.

When, around 10-days of age, pups become more mobile and are able to venture outside the nest[32], odor avoidance learning emerges, suggesting the appearance of learning adapted for exploring outside the nest. The basolateral amygdala is required for this infant fear conditioning, as it is in adult fear learning [26, 33-45] [29, 31]. Importantly, a causal relationship has been established for the amygdala in the emergence of fear conditioning since temporarily silencing the amygdala with the GABA agonist muscimol abolishes fear conditioning in these slightly older pups [29]. Importantly, the amygdala receives information about shock and odor prior to the emergence of fear conditioning[46], although the amygdala fails to respond in a manner that supports aversion learning. Indeed, it is this amygdala input in PN8 pups that is likely supporting the amygdala neurotransmitter and gene expression seen in the present data

Corticosterone is required for pups' amygdala-dependent fear learning.

Pups require corticosterone for the amygdala to be engaged in fear learning[29, 47], which is in sharp contrast to fear learning in adults where corticosterone only modulates learning[48-53]. Indeed, it is the appearance of mature corticosterone processes, rather than amygdala development that regulates the developmental emergence of the amygdala-dependent fear learning[29, 47]. A causal relationship between corticosterone level, amygdala and fear learning has been established: increasing amygdala corticosterone levels in very young pups produces fear learning; blocking amygdala corticosterone in older pups prevents fear learning.

Enduring effect of infant odor learning.

Memories of those odors conditioned in infancy, including those paired with shock, are retained into adulthood. When presented to adults, the odors enhance male and female sexual behavior [2, 54-57]; reduce adult fear conditioning [57, 58] and normalize performance in a forced swim tests (Sevelinges, Mouly & Sullivan, submitted). Thus, the positive valence of these preferred odors lasts into adulthood and modulates behavior in a manner consistent with that valence.

Supplementary Methods

Subjects. The subjects were Long-Evan (Harlan, Indiana) male and female 8- and 12-day-old rat pups born and breed at the University of Oklahoma. Animals were housed in polypropylene cages (34 X 29 X 17 cm) with ample pine bedding in a temperature

(20°C) and light (6:00 A.M. to 6:00 P.M.) controlled room. Food and water were available *ad libitum.* The day of parturition was considered 0-days-old and litters were culled to 12 at either 1- or 2-days-old. To avoid litter effects, no more than one male and one female from each litter were used in each experimental/test condition. All procedures were approved by the University of Oklahoma Institutional Animal Care and Use Committee and followed National Institutes of Health guidelines.

Systemic Corticosterone. 8-day old pups were injected twice (24 hrs and 30 min before conditioning) with either corticosterone (3.0 mg/kg, ip) or saline.

Odor-Shock Conditioning. Rat pups, 8 or 12 days old, were assigned to one of the three treatment groups, each lasting 45 min: 1) Paired odor-shock, 2) Unpaired odor-shock. Pups were trained in individual 600 ml plastic beakers and were adapted for 10 min to recover from experimental handling. During the 45 min conditioning session, pups received 11–30-sec presentations of a peppermint odor (CS) and a 1 sec 0.5 mA tail shock (US; Lafayette), with an intertrial interval of 4 min. Peppermint odor was delivered by a flow dilution olfactometer (2 L/min flow rate) at a concentration of 1:10 peppermint vapor. Paired odor-shock pups received 11 pairings of the 30 sec odor with shock overlapping during the last 1 sec of the odor presentation. Unpaired odor-shock pups received the shock 2 min after each odor presentation[29, 31, 47].

To verify learning during the training session, pup behavior was monitored for 20 sec before the odor and the first 20 sec during the odor presentation to construct an acquisition curve. Pups were also observed during the 1 sec shock[29, 31, 47]. A behavioral rating scale was used that recorded the number of limbs moving (0= no movement of the extremities; 5= movement of all 5 extremities including the head), and specific behaviors (i.e. head up for response to odor, wall climbing and vocalization to shock)[59].

Y-maze. The day following conditioning, pups were tested in a Y-maze. This test required pups to choose between two arms of a Plexiglas Y-maze (start box: 8.5 cm width, 10 cm length, 8 cm height; choice arms: 8.5 X 24 X 8 cm), one arm containing the 20 µl of the peppermint odor on a KimWipe placed at the end of the alley and the other containing the familiar odor of 20 ml of clean pine shavings placed at the end of the alley. Two doors separated the start box and the alleys. A pup was placed in the start box for 5 sec before the door to each alley was opened. Each pup was given 60 sec to

choose an arm. A response was considered a choice when a pup's entire body was past the entrance to the arm[29, 31, 47, 60].

Amygdala Dissection. Immediately after conditioning, the amygdala was dissected on ice. Dissection included bilateral localization of the amygdala using the ventral hippocampus and putamen landmark for the rostral and caudal cuts. A coronal view permitted localization of the rhinal fissure for the dorsal cut. The medial cut included a 45° cut from the optic chiasm and the removal of the lateral overlying cortical tissue. Amygdala removal was verified by histological verification of remaining tissue.

Microarrays/PCR. Biological replicates (n=3-8) of bilateral amygdalas were assayed with the Affymetrix 230A chip. Each animal represents a single data point. Comparisons were between paired and unpaired conditions.

Frozen harvested amygdala sections were ground into a powder by pestle in eppendorf tubes. Total RNA was isolated from the frozen tissue using Trizol Reagent and prepared and labeled using standard protocols. Fifteen μg of labeled cRNA sample were hybridized onto the microarrays, using standard protocols with the Affymetrix microarray oven and fluidics station at the Columbia University Genome Center. The hybridization pattern on the array was obtained by laser scanning into a high-resolution image and fluorescence intensity data was automatically stored in a raw file. To reduce the influence of technical variability, samples for each condition were always prepared and hybridized at the same time. Preprocessing, background correction and normalization (RMA) were done by GCRMA (http://www.bioconductor.org). Differential expression between paired and unpaired groups for each of the three conditions (8 day old; 8 day old CORT treated; 12 day old) was determined on logged (base 2) data by Ranked Products [61]. Correction for multiple testing was with False Detection Rate as described in the reference. We present the probes, their fold change and corrected probabilities in Tables 2 to 4 (to FDR $p < .30$).

SUPPLEMENTARY TABLE 2. Analysis of 8 day old pups: Paired vs. unpaired conditions

GeneID	Nucleotide	Title	Symbol	P-val.	Fold
1368601_at	NM_012694	solute carrier family 6, member 3	Slc6a3	0.000	-3.49
1368785_a_at	NM_019334	paired-like homeodomain transcription factor 2	Pitx2	0.000	-2.29
1374684_at	AI228978	R. norvegicus transcribed sequences	---	0.000	-4.89
1375535_at	AI103917	R. norvegicus transcribed sequences	---	0.000	-4.51
1387022_at	NM_022407	aldehyde dehydrogenase family 1, member A1	Aldh1a1	0.000	-2.03
1387075_at	NM_012740	tyrosine hydroxylase	Th	0.000	-3.32
1389712_at	BE117335	R. norvegicus transcribed sequences	---	0.000	-2.64
1369132_at	M97381	solute carrier family 18, member 2	Slc18a2	0.001	-2.26
1368802_at	NM_012625	pro-melanin-concentrating hormone	Pmch	0.002	-2.43
1368064_a_at	U31884	dopa decarboxylase	Ddc	0.004	-1.95
1370556_at	M24104	vesicle-associated membrane protein 1	Vamp1	0.008	-1.76
1377434_at	BG374415	R. norvegicus transcribed sequence with weak similarity to protein ref:NP_113621.1 (H.sapiens) membrane-type frizzled-related protein [Homo sapiens]	---	0.010	1.83
1368479_at	M35077	dopamine receptor 1A	Drd1a	0.017	1.76
1376755_at	BF419646	R. norvegicus transcribed sequences	---	0.019	1.91
1368478_at	NM_012546	dopamine receptor 1A	Drd1a	0.020	2.12
1372208_at	AA942959	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.020	2.01
1389989_at	BF397805	R. norvegicus transcribed sequence with strong similarity to protein pir:I38614 (H.sapiens) I38614 helicase II - human	---	0.020	1.75
1372823_at	BE117126	R. norvegicus transcribed sequences	---	0.022	-1.67
1367794_at	NM_012488	alpha-2-macroglobulin	A2m	0.022	-1.65
1368858_at	L21698	UDP-glucuronosyltransferase 8	Ugt8	0.023	-1.62
1370981_at	BE118450	R. norvegicus transcribed sequence with strong similarity to protein sp:P48443 (H.sapiens) RXRG_HUMAN Retinoic acid receptor RXR-gamma	---	0.024	1.58
1371679_at	BE113393	R. norvegicus transcribed sequences	---	0.024	-1.56
1387241_at	NM_031696	G protein-coupled receptor 88	Gpr88	0.028	1.77
1373257_at	AI412969	R. norvegicus transcribed sequence with moderate similarity to protein ref:NP_057384.1 (H.sapiens) cyclic AMP-regulated phosphoprotein, 21 kD [Homo sapiens]	---	0.034	1.83
1378038_at	BF393884	R. norvegicus transcribed sequences	---	0.034	-1.81
1383767_at	AW524430	R. norvegicus transcribed sequences	---	0.035	1.33
1368300_at	NM_053294	adenosine A2a receptor	Adora2a	0.037	1.59
1368348_at	NM_013034	solute carrier family 6, member 4	Slc6a4	0.049	-1.53
1368061_at	NM_031742	potassium voltage-gated channel, subfamily H (eag-related), member 1	Kcnh1	0.058	1.57

1377174_at	AW434982	R. norvegicus transcribed sequence with weak similarity to protein ref:NP_003903.1 (H.sapiens) myotubularin related protein 2 [Homo sapiens]	---	0.061	-1.43
1389986_at	AI008409	synaptic vesicle glycoprotein 2 b	Sv2b	0.062	1.14
1368677_at	NM_012513	brain derived neurotrophic factor	Bdnf	0.062	-1.50
1369731_at	AF102854	membrane-associated guanylate kinase-interacting protein	LOC59322	0.062	1.35
1388986_at	AI598339	R. norvegicus transcribed sequences	---	0.074	1.33
1373188_at	AI137995	sodium channel, voltage-gated, type IV, beta	Scn4b	0.078	1.59
1376278_at	BI282100	R. norvegicus transcribed sequences	---	0.079	-1.52
1368440_at	NM_017216	solute carrier family 3, member 1	Slc3a1	0.081	1.17
1390227_at	AI101017	R. norvegicus transcribed sequence with weak similarity to protein sp:P48539 (H.sapiens) PE19_HUMAN Brain specific polypeptide PEP-19 (Brain specific antigen PCP-4) (Purkinje cell protein 4)	---	0.082	1.68
1375751_at	BE098727	R. norvegicus transcribed sequence with weak similarity to protein pir:PIHUB6 (H.sapiens) PIHUB6 salivary proline-rich protein precursor PRB1 (large allele) - human	---	0.083	1.29
1370432_at	M72711	POU domain, class 3, transcription factor 1	Pou3f1	0.085	1.29
1398245_at	NM_031688	synuclein, gamma	Sncg	0.093	-1.56
1390722_at	AW531272	R. norvegicus transcribed sequences	---	0.108	1.04
1369131_at	NM_013031	solute carrier family 18, member 2	Slc18a2	0.117	-1.57
1390539_at	BF285569	R. norvegicus transcribed sequences	---	0.121	-1.09
1376967_at	AI176941	R. norvegicus transcribed sequences	---	0.139	1.39
1390649_at	AW520784	R. norvegicus transcribed sequences	---	0.148	-1.05
1367731_at	NM_030987	guanine nucleotide binding protein, beta 1	Gnb1	0.153	1.21
1373538_at	AW525342	R. norvegicus transcribed sequence with strong similarity to protein sp:O94782 (H.sapiens) UBP1_HUMAN Ubiquitin carboxyl-terminal hydrolase 1 (Ubiquitin thiolesterase 1) (Ubiquitin-specific processing protease 1) (Deubiquitinating enzyme 1) (hUBP)	---	0.154	1.44
1387693_a_at	M95413	glycine transporter 1	Glyt1	0.160	-1.56
1383059_a_at	BE112067	R. norvegicus similar to testis-specific gene (LOC361202), mRNA	---	0.166	-1.18
1369248_a_at	AF304333	baculoviral IAP repeat-containing 4	Birc4	0.166	-1.09
1387348_at	BE113270	insulin-like growth factor-binding protein 5	Igfbp5	0.167	-1.25
1389211_at	AA943808	R. norvegicus transcribed sequences	---	0.168	1.61
1371026_at	AY057064	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 4	Ppfia4	0.168	-1.30
1369297_at	NM_057116	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), gamma isoform	Ppp2r2c	0.170	-1.36
1369129_at	NM_019211	RAS guanyl releasing protein 1	Rasgrp1	0.172	1.54
1377316_at	BF397728	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.174	1.28

1372934_at	AI104146	R. norvegicus similar to 1700019E19Rik protein (LOC299209), mRNA	---	0.176	1.34
1371062_at	L34821	aldehyde dehydrogenase family 5, subfamily A1	Aldh5a1	0.178	1.53
1371208_at	AF000898	nucleoporin p58	p58/p45	0.188	-1.11
1368708_a_at	NM_012547	dopamine receptor 2	Drd2	0.192	1.44
1367816_at	NM_133621	global ischemia induced protein GIIG15B	GIIg15b	0.194	-1.41
1376017_at	BF390554	R. norvegicus similar to WD repeat domain 17 (LOC361188), mRNA	---	0.199	1.49
1370333_a_at	M15481	insulin-like growth factor 1	Igf1	0.200	-1.25
1376311_at	BM391312	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.201	-1.42
1387889_at	AI233882	folate receptor 1 (adult)	Folr1	0.201	1.36
1370669_a_at	AB027156	phosphodiesterase 10A	Pde10a	0.208	1.37
1392592_at	AI137045	R. norvegicus transcribed sequences	---	0.208	-1.45
1372481_at	AI102873	R. norvegicus transcribed sequence with weak similarity to protein pir:I67604 (H.sapiens) I67604 CD34 antigen short splice form precursor - human	---	0.209	1.26
1368641_at	NM_053402	wingless-type MMTV integration site family, member 4	Wnt4	0.210	-1.33
1377534_at	BE097305	R. norvegicus transcribed sequence with strong similarity to protein ref:NP_067277.1 (M.musculus) hypothetical serine/threonine protein kinase [Mus musculus]	---	0.210	1.29
1370544_at	AF335571	echinoderm microtubule associated protein like 2	Eml2	0.211	1.21
1389868_at	AA850780	R. norvegicus transcribed sequences	---	0.211	1.31
1368312_at	M25649	oxcytocin	Oxt	0.212	1.32
1372958_at	BE111986	R. norvegicus transcribed sequences	---	0.212	1.21
1371059_at	AW919085	protein kinase, cAMP-dependent, regulatory, type 2, alpha	Prkar2a	0.213	-1.29
1374065_at	BG378920	R. norvegicus transcribed sequences	---	0.213	1.11
1371960_at	AI230548	R. norvegicus transcribed sequences	---	0.213	1.24
1384101_at	BM388523	R. norvegicus transcribed sequence with strong similarity to protein sp:O00401 (H.sapiens) WASL_HUMAN Neural Wiskott-Aldrich syndrome protein (N-WASP)	---	0.214	1.48
1379640_at	AI578083	R. norvegicus transcribed sequences	---	0.214	-1.39
1368887_at	NM_019161	cadherin 22	Cdh22	0.214	-1.24
1387450_at	NM_012671	transforming growth factor alpha	Tgfa	0.214	1.31
1375043_at	BF415939	R. norvegicus transcribed sequence with strong similarity to protein sp:P01100 (H.sapiens) FOS_HUMAN Proto-oncogene protein c-fos (Cellular oncogene fos) (G0/G1 switch regulatory protein 7)	---	0.215	1.41
1377006_at	AA875047	R. norvegicus similar to CCT (chaperonin containing TCP-1) zeta subunit (LOC303526), mRNA	---	0.215	1.10

1374899_at	BM383043	R. norvegicus similar to hypothetical protein 4933417N17 (LOC290877), mRNA	---	0.215	-1.45
1372280_at	BI295982	R. norvegicus transcribed sequence with strong similarity to protein pir:T46507 (H.sapiens) T46507 hypothetical protein DKFZp586M2121.1 - human (fragment)	---	0.216	1.52
1375468_at	AW252983	ATP-binding cassette, sub-family C (CFTR/MRP), member 5a	Abcc5a	0.216	-1.48
1369822_at	NM_022264	c-kit receptor tyrosine kinase	Kit	0.217	-1.32
1370751_at	U77931	---	---	0.217	1.19
1369753_at	M63334	calcium/calmodulin-dependent protein kinase IV	Camk4	0.217	1.07
1374879_x_at	AI228249	R. norvegicus transcribed sequences	---	0.218	1.10
1376842_at	BF395964	R. norvegicus transcribed sequences	---	0.218	1.22
1390192_at	BF406261	R. norvegicus transcribed sequence with moderate similarity to protein ref:NP_077306.1 (H.sapiens) hypothetical protein MGC4365 [Homo sapiens]	---	0.219	-1.51
1379281_at	AA892798	uterine sensitization-associated gene 1 protein	Usag1	0.220	1.16
1389994_at	BE104268	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.220	1.12
1387720_at	NM_134377	calsyntenin 2	Cstn2	0.221	-1.34
1388569_at	AI179984	alpha-2 antiplasmin	Dmrs91	0.221	-1.21
1375187_at	BF281701	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.222	-1.26
1370606_at	U22830	purinergic receptor P2Y, G-protein coupled 1	P2ry1	0.223	1.35
1388689_at	AI170755	R. norvegicus transcribed sequence with moderate similarity to protein sp:P14621 (H.sapiens) ACYM_HUMAN Acylphosphatase, muscle type isozyme (Acylphosphate phosphohydrolase)	---	0.224	1.32
1374283_at	BF419505	R. norvegicus transcribed sequence with strong similarity to protein ref:NP_004450.1 (H.sapiens) fetal Alzheimer antigen [Homo sapiens]	---	0.225	-1.12
1388398_at	BI282024	R. norvegicus transcribed sequence with moderate similarity to protein sp:P12750 (H.sapiens) RS4_HUMAN 40S ribosomal protein S4, X isoform (Single copy abundant mRNA protein) (SCR10)	---	0.227	-1.35
1369610_at	NM_021851	lin-7-C	Lin7c	0.228	-1.24
1376280_at	AA799789	R. norvegicus transcribed sequences	---	0.230	-1.29
1375648_at	BE113026	R. norvegicus transcribed sequences	---	0.232	1.43
1375174_at	BI296653	R. norvegicus transcribed sequences	---	0.234	-1.19
1376636_at	BE111972	R. norvegicus transcribed sequences	---	0.234	1.49
1399109_at	BI281673	R. norvegicus transcribed sequences	---	0.234	1.33
1387541_at	NM_031653	chondroitin sulfate proteoglycan 3	Cspg3	0.234	1.10

1389474_at	AA818380	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.235	-1.18
1368957_at	NM_024138	guanine nucleotide binding protein, gamma 7	Gng7	0.236	1.42
1387274_at	NM_012943	distal-less homeobox 5	Dlx5	0.237	1.52
1387425_at	NM_031779	amyloid beta (A4) precursor protein-binding, family A, APBA1: amyloid beta (A4) precursor protein-binding, family A, member 1 (X11)	Apba1	0.238	1.55
1376729_at	AI231781	R. norvegicus transcribed sequence with strong similarity to protein ref:NP_036228.1 (H.sapiens) adaptor protein containing pH domain, PTB domain and leucine zipper motif [Homo sapiens]	---	0.238	1.36
1373171_at	BI294854	R. norvegicus transcribed sequences	---	0.238	1.50
1370228_at	AA945178	Transferrin	Tf	0.239	-1.16
1369818_at	NM_012957	gamma-aminobutyric acid receptor, subunit beta 2	Gabrb2	0.239	1.28
1377720_x_at	AA892765	---	---	0.240	1.31
1375676_at	AA956897	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.242	1.34
1387349_at	NM_013028	short stature homeobox 2	Shox2	0.243	-1.38
1368867_at	BF281131	GERp95	Gerp95	0.243	1.28
1370770_s_at	AF071205	Kit ligand	Kitl	0.243	-1.09
1388216_at	L03386	Olf-1/EBF associated Zn finger protein Roaz	Roaz	0.244	-1.26
1370705_at	AF277900	zinc finger protein HIT-4	Hit4	0.244	1.18
1368438_at	NM_022236	phosphodiesterase 10A	Pde10a	0.245	1.32
1387170_at	NM_053824	casein kinase II, alpha 1 polypeptide	Csnk2a 1	0.246	1.34
1375217_at	BI296680	R. norvegicus transcribed sequences	---	0.247	-1.48
1376709_at	BM388442	R. norvegicus similar to RIKEN cDNA 4933419D20 (LOC295455), mRNA	---	0.247	-1.21
1389068_at	BI288819	R. norvegicus transcribed sequence with moderate similarity to protein pir:T12539 (H.sapiens) T12539 hypothetical protein DKFZp434J154.1 - human	---	0.249	1.44
1388072_at	AB010154	SH3-binding kinase	Sbk	0.249	-1.19
1383263_at	BG664221	R. norvegicus transcribed sequence with moderate similarity to protein pir:B35272 (H.sapiens) B35272 osteoinductive factor - human	---	0.250	-1.35
1374655_at	BG378095	R. norvegicus transcribed sequences	---	0.251	-1.10
1379594_at	AW524408	R. norvegicus transcribed sequences	---	0.251	-1.24
1368990_at	NM_012940	cytochrome P450, subfamily 1B, polypeptide 1	Cyp1b1	0.252	-1.28
1368263_a_at	X90402	myelin-associated oligodendrocytic basic protein	Mobp	0.253	-1.44
1387549_at	NM_024361	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1	Ndst1	0.254	-1.17
1369628_at	BG672437	synaptic vesicle glycoprotein 2 b	Sv2b	0.255	-1.61

1387646_a_at	NM_022210	Max	Max	0.255	**-1.04**
1387406_at	NM_017293	kinase interacting with leukemia-associated gene	Kist	0.255	**-1.06**
1376704_a_at	BM390112	R. norvegicus transcribed sequence with weak similarity to protein sp:Q9HAY2 (H.sapiens) MGF1_HUMAN Melanoma-associated antigen F1 (MAGE-F1 antigen)	---	0.257	**-1.13**
1387844_at	NM_032613	LIM and SH3 protein 1	Lasp1	0.259	**-1.28**
1390383_at	BI285616	R. norvegicus transcribed sequence with weak similarity to protein sp:O60664 (H.sapiens) TI47_HUMAN Cargo selection protein TIP47 (47 kDa mannose 6-phosphate receptor-binding protein) (47 kDa MPR-binding protein) (Placental protein 17)	---	0.260	**-1.35**
1369344_at	NM_023975	TGF-beta resistance-associated protein	Trag	0.260	**-1.40**
1377921_at	AA875050	R. norvegicus transcribed sequence with strong similarity to protein sp:Q9NVF9 (H.sapiens) EKI2_HUMAN Ethanolamine kinase-like protein EKI2 (FLJ10761)	---	0.260	**-1.32**
1369135_at	NM_031667	synaptotagmin 11	Syt11	0.261	**-1.30**
1369754_a_at	NM_053295	calpastatin	Cast	0.261	**-1.41**
1370434_a_at	X89638	myelin-associated oligodendrocytic basic protein	Mobp	0.262	**-1.41**
1369590_a_at	NM_024134	DNA-damage inducible transcript 3	Ddit3	0.262	**-1.15**
1374921_at	BI274548	R. norvegicus transcribed sequence with weak similarity to protein pir:T45294 (H.sapiens) T45294 hypothetical protein KIAA1088 [imported] - human (fragment)	---	0.262	**-1.18**
1387341_a_at	NM_017026	myelin basic protein	Mbp	0.263	**-1.36**
1388357_at	BI282972	R. norvegicus transcribed sequence with weak similarity to protein sp:Q9UI56 (H.sapiens) P518_HUMAN Protein PRO0518	---	0.263	**-1.44**
1374276_at	BE104102	R. norvegicus transcribed sequences	---	0.264	**-1.10**
1373991_at	AI411366	R. norvegicus transcribed sequence with weak similarity to protein ref:NP_079268.1 (H.sapiens) hypothetical protein FLJ12547 [Homo sapiens]	---	0.264	**-1.30**
1388221_at	AY009158	solute carrier family 24, member 3	Slc24a3	0.265	**-1.17**
1375305_at	BI282028	R. norvegicus transcribed sequence with strong similarity to protein sp:Q15531 (H.sapiens) ST1B_HUMAN Syntaxin 1B	---	0.266	**-1.42**
1389754_at	AI555295	R. norvegicus transcribed sequence with weak similarity to protein ref:NP_477515.1 (H.sapiens) ubiquitin conjugating enzyme 6 [Homo sapiens]	---	0.266	**-1.09**
1374803_at	BG374219	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.266	**-1.26**
1376456_at	BE114264	R. norvegicus similar to nicotinamide mononucleotide adenylyltransferase 2 isoform 1; pyridine nucleotide adenylyltransferase 2; chromosome 1 open reading frame 15 (LOC289095), mRNA	---	0.266	**-1.43**
1371066_at	X89383	SNF related kinase	Snrk	0.268	**-1.23**

1375707_at	AA817993	---	---	0.269	**-1.26**
1375352_at	AI177506	R. norvegicus transcribed sequence with moderate similarity to protein sp:O60909 (H.sapiens) B4G2_HUMAN Beta-1,4-galactosyltransferase 2 (Beta-1,4-GalTase 2) (Beta4Gal-T2) (b4Gal-T2) (UDP-galactose:beta-N-acetylglucosamine beta-1,4-galactosyltransferase 2) (UDP-Gal:beta-GlcNAc beta-1,4-galactosyltransferase 2) [Includes: Lactose synthase A protein ; N-acetyllactosamine synthase (Nal synthetase); Beta-N-acetylglucosaminyl-glycopeptide beta-1,4-galactosyltransferase ; Beta-N-acetylglucosaminyl-glycolipid beta-1,4-galactosyltransferase]	---	0.269	**-1.34**
1392246_at	AA900645	R. norvegicus transcribed sequences	---	0.269	**-1.16**
1370052_at	NM_031081	3-phosphoinositide dependent protein kinase-1	Pdpk1	0.271	**1.19**
1387144_at	NM_030994	integrin alpha 1	Itga1	0.272	**-1.30**
1375422_at	AI710284	---	---	0.281	**1.24**

SUPPLEMENTARY TABLE 3. Analysis of 8 day old pups pretreated with CORT: Paired vs unpaired conditions

GeneID	Nucleotide	Title	Symbol	P-val.	Fold
1389699_at	AW433598	R. norvegicus transcribed sequences	---	0.000	1.90
1387075_at	NM_012740	tyrosine hydroxylase	Th	0.003	1.89
1372213_at	BM390487	R. norvegicus transcribed sequence with moderate similarity to protein pir:T46271 (H.sapiens) T46271 hypothetical protein DKFZp564P1263.1 - human	---	0.005	1.79
1372510_at	AI172302	R. norvegicus transcribed sequence with weak similarity to protein pir:S43056 (M.musculus) S43056 hypothetical protein - mouse	---	0.013	1.70
1368601_at	NM_012694	solute carrier family 6, member 3	Slc6a3	0.052	2.14
1373240_at	BI276935	R. norvegicus transcribed sequence with strong similarity to protein ref:NP_035433.1 (M.musculus) retinal short-chain dehydrogenase/reductase 1 [Mus musculus]	---	0.073	1.58
1388398_at	BI282024	R. norvegicus transcribed sequence with moderate similarity to protein sp:P12750 (H.sapiens) RS4_HUMAN 40S ribosomal protein S4, X isoform (Single copy abundant mRNA protein) (SCR10)	---	0.079	1.60
1370551_a_at	AB000817	semaphorin 6c	Sema6c	0.085	1.56
1370556_at	M24104	vesicle-associated membrane protein 1	Vamp1	0.123	1.52
1369030_at	NM_012635	pancreatic trypsin 1	Prss1	0.153	1.52
1379281_at	AA892798	uterine sensitization-associated gene 1 protein	Usag1	0.163	-1.99
1387189_at	NM_019230	solute carrier family 22, member 3	Slc22a3	0.165	-1.49
1374540_at	AA859235	R. norvegicus transcribed sequences	---	0.168	-1.43
1370428_x_at	AJ249701	RT1 class Ib gene(Aw2)	RT1Aw2	0.170	-1.69
1375707_at	AA817993	---	---	0.172	-1.55
1377020_at	BF416408	R. norvegicus transcribed sequence with weak similarity to protein sp:Q9Y487 (H.sapiens) VPP2_HUMAN Vacuolar proton translocating ATPase 116 kDa subunit A isoform 2 (V-ATPase 116-kDa isoform a2) (TJ6)	---	0.177	-1.56
1374684_at	AI228978	R. norvegicus transcribed sequences	---	0.180	-7.03
1375343_at	BE116572	R. norvegicus transcribed sequences	---	0.181	-1.55
1377366_at	BF389753	R. norvegicus transcribed sequences	---	0.185	1.41
1375826_at	BF410192	R. norvegicus transcribed sequences	---	0.186	1.45
1373831_at	AW920524	R. norvegicus transcribed sequence with moderate similarity to protein ref:NP_078786.1 (H.sapiens) hypothetical protein MGC4368 [Homo sapiens]	---	0.187	1.46
1368753_at	NM_031338	Ca+/Calmodulin-dependent protein kinase kinase beta (CaM-kinase kinase beta)	Camkk2	0.188	1.49
1376918_at	AI029930	R. norvegicus transcribed sequences	---	0.195	1.52
1369754_a_at	NM_053295	calpastatin	Cast	0.195	-1.49

1383336_at	BF549763	R. norvegicus transcribed sequence with moderate similarity to protein ref:NP_002678.1 (H.sapiens) pinin, desmosome associated protein [Homo sapiens]	---	0.198	**-1.47**
1375535_at	AI103917	R. norvegicus transcribed sequences	---	0.198	**-6.02**
1371917_at	AI411119	R. norvegicus transcribed sequence with moderate similarity to protein sp:O75648 (H.sapiens) TRMU_HUMAN tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase	---	0.200	**-1.49**
1389211_at	AA943808	R. norvegicus transcribed sequences	---	0.201	**-1.66**
1368785_a_at	NM_019334	paired-like homeodomain transcription factor 2	Pitx2	0.201	**-1.57**
1370697_a_at	AF056034	nexilin	LOC246172	0.205	**-1.75**
1389332_at	BF283404	R. norvegicus transcribed sequences	---	0.209	**-1.43**
1368478_at	NM_012546	dopamine receptor 1A	Drd1a	0.212	**-1.72**
1387889_at	AI233882	folate receptor 1 (adult)	Folr1	0.215	**-1.60**
1374563_at	BI301280	R. norvegicus transcribed sequences	---	0.220	**-1.48**
1376297_at	BE112202	R. norvegicus transcribed sequence with weak similarity to protein ref:NP_004783.1 (H.sapiens) peptidyl-prolyl isomerase G (cyclophilin G); Clk-associating RS-cyclophilin [Homo sapiens]	---	0.224	**1.44**
1375026_at	AI105369	R. norvegicus similar to CLN6 protein (LOC315746), mRNA	---	0.225	**-1.53**
1368300_at	NM_053294	adenosine A2a receptor	Adora2a	0.225	**-1.38**
1372208_at	AA942959	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.225	**-1.96**
1373081_at	AI105000	R. norvegicus transcribed sequences	---	0.233	**-1.41**
1367823_at	BF523128	tissue inhibitor of metalloproteinase 2	Timp2	0.234	**1.43**
1368479_at	M35077	dopamine receptor 1A	Drd1a	0.253	**-1.55**
1388848_at	AA891760	R. norvegicus transcribed sequence with moderate similarity to protein ref:NP_071934.1 (H.sapiens) hypothetical protein FLJ22056 [Homo sapiens]	---	0.254	**-1.45**
1387487_a_at	NM_053816	calcitonin receptor	Calcr	0.260	**-1.38**
1387241_at	NM_031696	G protein-coupled receptor 88	Gpr88	0.266	**-1.53**
1368957_at	NM_024138	guanine nucleotide binding protein, gamma 7	Gng7	0.271	**-1.56**
1389438_at	BE118454	R. norvegicus similar to testes development-related NYD-SP22 isoform 1 (LOC297999), mRNA	---	0.271	**-1.47**
1370301_at	U65656	matrix metalloproteinase 2 (72 KDa type IV collagenase)	Mmp2	0.275	**-1.49**
1374090_at	BI296363	R. norvegicus transcribed sequences	---	0.279	**1.39**
1374001_at	AA851818	R. norvegicus transcribed sequences	---	0.285	**1.38**
1374632_at	AI232697	R. norvegicus transcribed sequence with weak similarity to protein ref:NP_075383.1 (H.sapiens) hypothetical protein FLJ12517 [Homo sapiens]	---	0.289	**-1.36**

1373743_at	BI295567	R. norvegicus transcribed sequences	---	0.289	**-1.41**
1369332_a_at	NM_052829	Rim1 protein	Rim1	0.290	**1.41**
1368459_at	NM_024375	prepro bone inducing protein	Gdf10	0.295	**1.46**
1372280_at	BI295982	R. norvegicus transcribed sequence with strong similarity to protein pir:T46507 (H.sapiens) T46507 hypothetical protein DKFZp586M2121.1 - human (fragment)	---	0.297	**-1.10**
1367631_at	NM_022266	connective tissue growth factor	Ctgf	0.299	**-1.37**

SUPPLEMENTARY TABLE 4. Analysis of 12 day old pups: Paired vs unpaired conditions

GeneID	Nucleotide	Title	Symbol	P-val.	Fold
1367598_at	NM_012681	transthyretin	Ttr	0.010	**4.27**
1375664_at	BI294732	R. norvegicus similar to trinucleotide repeat containing 6; EDIE; GW182 autoantigen (LOC308971), mRNA	---	0.015	**2.55**
1375026_at	AI105369	R. norvegicus similar to CLN6 protein (LOC315746), mRNA	---	0.015	**2.13**
1368601_at	NM_012694	solute carrier family 6, member 3	Slc6a3	0.016	**2.25**
1379281_at	AA892798	uterine sensitization-associated gene 1 protein	Usag1	0.020	**2.60**
1387022_at	NM_022407	aldehyde dehydrogenase family 1, member A1	Aldh1a1	0.025	**1.77**
1371776_at	AA819268	R. norvegicus transcribed sequences	---	0.026	**2.60**
1386995_at	BI288701	B-cell translocation gene 2, anti-proliferative	Btg2	0.040	**1.82**
1387163_at	NM_013179	hypocretin	HcRt	0.049	**1.83**
1387488_a_at	L13041	calcitonin receptor	Calcr	0.059	**2.14**
1388985_at	AI012869	R. norvegicus transcribed sequence with weak similarity to protein sp:P20908 (H.sapiens) CA15_HUMAN Collagen alpha 1(V) chain precursor	---	0.060	**1.75**
1369625_at	AA891661	aquaporin 1	Aqp1	0.069	**1.74**
1370806_at	AF465614	hypothetical protein RMT-7	Rmt7	0.069	**1.80**
1369131_at	NM_013031	solute carrier family 18, member 2	Slc18a2	0.070	**1.80**
1370310_at	M33648	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Hmgcs2	0.070	**1.76**
1387075_at	NM_012740	tyrosine hydroxylase	Th	0.071	**1.30**
1370544_at	AF335571	echinoderm microtubule associated protein like 2	Eml2	0.071	**1.54**
1374320_at	AI717113	coagulation factor 5	F5	0.072	**1.77**
1368028_at	NM_012633	peripherin 1	Prph1	0.075	**1.65**
1387889_at	AI233882	folate receptor 1 (adult)	Folr1	0.078	**1.87**
1368585_at	NM_017110	cocaine and amphetamine regulated transcript	Cart	0.079	**1.91**
1368312_at	M25649	oxcytocin	Oxt	0.113	**1.85**
1388155_at	BI286012	keratin complex 1, acidic, gene 18	Krt1-18	0.120	**1.59**
1376692_at	BM392321	R. norvegicus transcribed sequence with strong similarity to protein ref:NP_073577.1 (H.sapiens) homeodomain-interacting protein kinase 2 [Homo sapiens]	---	0.120	**1.42**
1368802_at	NM_012625	pro-melanin-concentrating hormone	Pmch	0.121	**1.81**
1376873_at	AI227829	R. norvegicus transcribed sequence with strong similarity to protein pir:A37873 (H.sapiens) A37873 cerebellin precursor - human	---	0.121	**1.80**
1388742_at	AA945877	R. norvegicus transcribed sequences	---	0.122	**1.45**
1377434_at	BG374415	R. norvegicus transcribed sequence with weak similarity to protein ref:NP_113621.1 (H.sapiens) membrane-type frizzled-related protein [Homo sapiens]	---	0.123	**1.41**
1369614_at	NM_133410	RAP2B, member of RAS oncogene family	Rap2b	0.123	**-1.97**
1368082_at	NM_017048	solute carrier family 4, member 2	Slc4a2	0.130	**1.55**
1375777_at	BF402633	R. norvegicus transcribed sequences	---	0.133	**-2.31**
1376129_at	BE108174	R. norvegicus transcribed sequences	---	0.134	**-2.76**
1368300_at	NM_053294	adenosine A2a receptor	Adora2a	0.143	**-1.75**
1369248_a_at	AF304333	baculoviral IAP repeat-containing 4	Birc4	0.143	**-1.49**

1370092_at	NM_012757	MAS1 oncogene	Mas1	0.145	-1.57
1371442_at	BI282904	oxygen regulated protein (150kD)	Orp150	0.154	1.55
1374280_at	AA817812	R. norvegicus transcribed sequences	---	0.156	1.51
1368751_at	NM_031778	Shab-related delayed-rectifier K+ channel (Kv9.3)	Kcns3	0.156	1.56
1387410_at	U72345	nuclear receptor subfamily 4, group A, member 2	Nr4a2	0.158	1.47
1390722_at	AW531272	R. norvegicus transcribed sequences	---	0.160	-1.35
1389734_x_at	BI282965	RT1 class Ib gene(Aw2)	RT1Aw2	0.173	1.40
1389996_at	AI406369	R. norvegicus transcribed sequences	---	0.185	-1.76
1374958_at	AA819640	R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only	---	0.198	-1.63
1386941_at	NM_022401	R. norvegicus transcribed sequence with strong similarity to protein ref:NP_000436.1 (H.sapiens) plectin 1, intermediate filament binding protein, 500kD [Homo sapiens]	---	0.204	-1.81
1369663_at	NM_022936	cytosolic epoxide hydrolase	Ephx2	0.205	-1.69
1368479_at	M35077	dopamine receptor 1A	---	0.213	1.40
1373097_at	BF282125	R. norvegicus transcribed sequence with weak similarity to protein ref:NP_286085.1 (E. coli) beta-D-galactosidase [Escherichia coli O157:H7 EDL933]	---	0.218	-1.58
1377069_at	BG379055	---	Syt12	0.219	1.46
1376096_a_at	BE108246	R. norvegicus transcribed sequences	---	0.219	-1.54
1368438_at	NM_022236	phosphodiesterase 10A	---	0.222	1.45
1376749_at	AA945955	R. norvegicus transcribed sequence with moderate similarity to protein pir:B35272 (H.sapiens) B35272 osteoinductive factor - human	Scn4b	0.223	1.39
1370935_at	AI105205	transporter-like protein	---	0.224	1.35
1388060_at	U71294	synaptotagmin XII	LOC171396	0.225	-1.57
1376843_at	BE118651	R. norvegicus transcribed sequence with strong similarity to protein prf:2109341A (H.sapiens) 2109341A Ser/Thr kinase receptor:ISOTYPE=type II [Homo sapiens]	Pcyt1a	0.229	1.46
1376419_at	BE116226	R. norvegicus transcribed sequences	---	0.229	-1.54
1370981_at	BE118450	R. norvegicus transcribed sequence with strong similarity to protein sp:P48443 (H.sapiens) RXRG_HUMAN Retinoic acid receptor RXR-gamma	Camk2a	0.230	1.38
1371595_at	BM384301	R. norvegicus transcribed sequence with weak similarity to protein pir:A43932 (H.sapiens) A43932 mucin 2 precursor, intestinal - human (fragments)	---	0.232	-2.15
1383205_at	BI288833	R. norvegicus transcribed sequences	---	0.233	1.41
1373188_at	AI137995	sodium channel, voltage-gated, type IV, beta	Ddx20	0.234	-1.53
1377236_at	BF405622	R. norvegicus transcribed sequences	Camk4	0.239	-1.62
1373062_at	BM388650	sulfatase FP	---	0.241	1.59
1369656_at	M36071	phosphate cytidylyltransferase 1, choline, alpha isoform	Nup155	0.283	-1.63
1369919_at	NM_019194	thyrotroph embryonic factor	Cacna1a	0.284	1.47
1390719_at	BI294889	R. norvegicus transcribed sequences	---	0.284	1.44

1375139_at	BF406295	R. norvegicus transcribed sequences	---	0.285	-1.66
1370669_a_at	AB027156	phosphodiesterase 10A	---	0.285	1.37
1377823_at	AW531363	R. norvegicus transcribed sequences	---	0.288	1.41
1388187_at	BM384558	calcium/calmodulin-dependent protein kinase II alpha subunit	Sncg	0.289	-1.54
1390501_at	BE101157	R. norvegicus transcribed sequences	---	0.290	1.42
1386939_a_at	AF051527	calcium channel, voltage-dependent, alpha 1A subunit	---	0.292	1.15

Tables 2-4. These tables show the results for paired conditions vs. unpaired conditions for 8 day old; 8 day old CORT treated and 12 day old animals. Probes are defined by Affymetrix probe ID's, NCBI reference sequences, and gene symbols. The probabilities listed are corrected by the method of Benjamini and Hochberg as implemented in Ranked Products methods[61]. We show up to corrected p<.30. Fold change are given in the last column. Red is upregulated whereas green is downregulated.

PCR. For PCR, RNA samples were from the same tissue samples (paired versus unpaired) as used for the microarray experiments. We used the SYBR-green detection system. This method allows the detection of product amplification during the PCR reaction by measuring the on-line incorporation of fluorescence that is incorporated into the amplicons. In the linear range of amplification, the amount of PCR products are directly correlated to relative levels of mRNA and can therefore be used to compare expression levels either between different genes in a same sample or between same genes across different samples. Samples are normalized to internal controls (GADPH). An Opticon real-time PCR machine (MJ Research, Waltham, MA) was used, using universal PCR conditions (65°C to 59°C touch-down, followed by 35 cycles [15' at 95°C, 10' at 59°C and 10' at 72°C]). 150 pg of cDNA was amplified in 20 µl reactions [0.3X Sybr-green, 3 mM MgCl2, 200 µM dNTPs, 200 µM primers, 0.5 unit Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA)]. Results were calculated as relative intensity compared to GADPH by the Delta Delta Ct method. PCR primers are listed in Table 5.

GADPH controls. To assess if the manipulations altered the GAPDH controls, we assessed the number of cycles for GAPDH alone, with the assumption that variation should be random unless altered systematically by the treatments. The results are shown in Table 6. There were no differences related to treatment and all cycle values were very similar. We ran also 18S controls for the 12-day old pups and again found no differences (data not shown).

SUPPLEMENTARY TABLE 5. Primers for PCR				
Code	**Name of Gene**	**Sense**	**Anti-Sense**	**Size & Location**
1368601	solute carrier family 6 (DAT), member 3 (Slc6a3)	caccatgtgtctccctga	ggtaaaccggagccttct	147 (324-470)
1368601-2	solute carrier family 6 (DAT), member 3 (Slc6a3)	ctggctttagttcctggagt	accctcgtgccaatgtat	161 (230-390)
1387075	tyrosine hydroxylase	tgtactttgtgtccgagagc	ccagtgtgtacgggtcaa	105 (120-224)
1387075-2	tyrosine hydroxylase	ggctgctgtcttcctacg	ggcatagttcctgagcttgt	173 (3-175)
1387022	aldehyde dehydrogenase, family 1, member A1	ctccagtccttatcccagaa	gtcctcctcaccaaatgagt	119 (266-384)
1387022-2	aldehyde dehydrogenase, family 1, member A1	tccagtccttatcccagaat	gtcacagagggacagagctta	150 (267-416)

Note: These are the primers for each of three dopamine related genes differentially regulated by corticosterone at 8 days of age. Two different primers, taken from Affymetrix and other published sequences were used for each gene.

SUPPLEMENTARY TABLE 6. GADPH cycles					
8day			**12 Day**		
Exp. Condition	**Mean**	**SEM**	**Exp. Condition**	**Mean**	**SEM**
Paired-Sal.	15.186	0.154	Paired	15.029	0.279
Unpair-Sal.	15.013	0.126	Unpaired	15.015	0.137
Paired -Cort.	15.016	0.198			
Unpair-Cort.	15.146	0.218			

Note: Each sample was run in triplicate and averaged for each of three 3 separate animals. Means shown here are of the averaged triplicates. There is no difference between treatment groups. Likewise in a limited number of animals, 18S was used as a control and likewise showed no significant effects of treatment.

Microdialysis / HPLC procedures. Two days before conditioning, pups were anesthetized (isofluorane) and placed in a stereotaxic apparatus adapted for infant rats. Stainless steel cannulae (30-gauge tubing) were implanted unilaterally (caudal -0.90mm; lateral ±4.50mm from bregma; lowered 6.0mm) aimed at the basolateral nucleus of the amygdala through a hole drilled in the overlying skull. Following recovery from surgery (approximately 30 to 60 minutes), pups were returned to the nest[29, 47, 62].

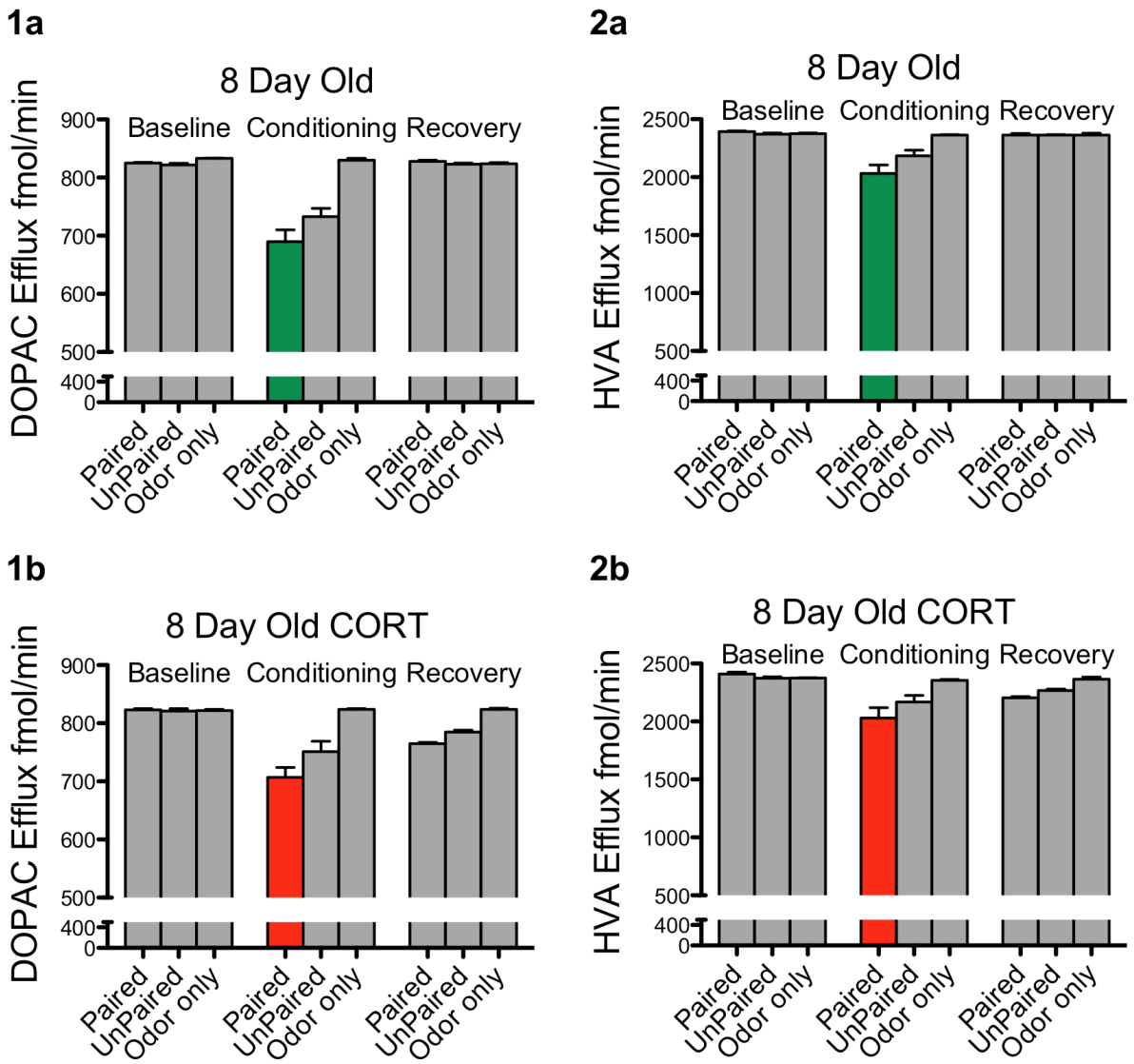
On the day of the experiment, pups were placed in a 27cm diameter acrylic circular cage (EICOM corp., Kyoto, Japan) and were able to move freely. The microdialysis probe (A-I-8-02, 8mm length, 2mm membrane, 220μm diameter; EICOM corp., Kyoto, Japan) was inserted into the guide cannula 20 minutes before collection. The probes were perfused with artificial cerebrospinal fluid (ACSF; 147mM NaCl, 2.7mM KCl, 1.2mM CaCl2, 0.85mM MgCl2) at a flow rate of 1.5μl/min. Dialysate was collected automatically every 10 minutes in a refrigerated (4°C) microfraction collector (EICOM corp., Kyoto, Japan; EFC-82) in which every vial contained 2μl of 12.5mM perchloric acid/ 250μM EDTA. After a 2 hour baseline collection, awake pups were given either 11 paired odor-0.5mA shock, 11 unpaired odor-shock or 11 odor only presentations during the 45 min conditioning, followed by a recovery period collection of at least one hour. After completion of the experiment, dialysate samples were immediately stored at -80°C until HPLC analysis[62]. All neurochemicals (including NE and 5-HT not shown here) were assayed in the same dialysate.

Dopamine and its metabolites were assessed by high-pressure liquid chromatography with electrochemical detection (HPLC-EC). HPLC-EC consisted of a 150 x 2.1mm SC-5ODS, 5μm particle column (EICOM corp., Kyoto Japan). Mobile phase (0.1M citric acid, 0.25mM octyl sulfate sodium salt, 0.5mM EDTA, 0.085 tryethylamine, and 6% acetonitrile, pH 2.4) was delivered at 0.23ml/ min by a EICOM EP-300 pump were detected with a graphite carbon detector electrode maintained at +0.75V relative to an Ag/AgCl reference electrode. Neurochemical concentrations were estimated using chromatographic peak areas and calibration curves obtained with standard mixtures of known monoamine compounds. Calibration of dopamine was performed daily and standard calibration curves constructed. Furthermore, during the course of dialysate autoinjection fractions, a standard mixture was injected every fifth sample to monitor and correct calibration curves[61]. Probe placements are shown in Supplementary Figure 3.

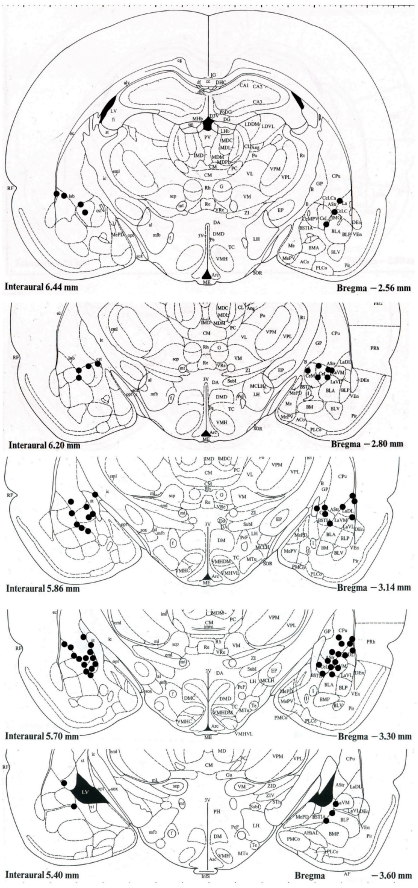
Amygdala dopamine /receptor antagonist infusion. At 6-days-old, pups were anesthetized by inhalation with isoflurane and placed in an adult stereotaxic apparatus modified for use with infants. Stainless steel cannulas (30-gauge tubing) were implanted bilaterally in the amygdaloid complex through holes drilled in the overlying skull. The bilateral cannula were implanted (caudal 0.80mm; lateral ±3.00mm from bregma, lowered 5.0mm from skull surface) and fixed to the skull with dental cement. To ensure cannula patency, guide wires were placed in the lumen of the tubing and Nailbiter (bitter tasting product to discourage nail biting) wa applied to the pup's cannula to discourage the mother from biting the surgical area. Following a 30 min recovery, the pup was returned to the nest until conditioning at 8-days-old. For conditioning, pups were placed in individual 600-ml plastic beakers. Their bilateral cannulae were attached via PE10 tubing to a Harvard syringe pump driving two Hamilton microliter syringes[29, 47]. The cannulae were filled (16 sec at 0.5 µl/min) with either a dopamine receptor antagonist (cis-(Z)-flupenthixol dihydrochloride 20 µg; Sigma), dopamine (3-6 µg, Sigma) or saline[62-64]. During the first 20-minutes of the conditioning period, pups received drug or control solution infused at 0.1µl/min, for a total infusion volume of 2.0 µl as previously described[29, 47]. Following conditioning, pups were disconnected from the syringe pump and returned to the nest until testing, the following day.

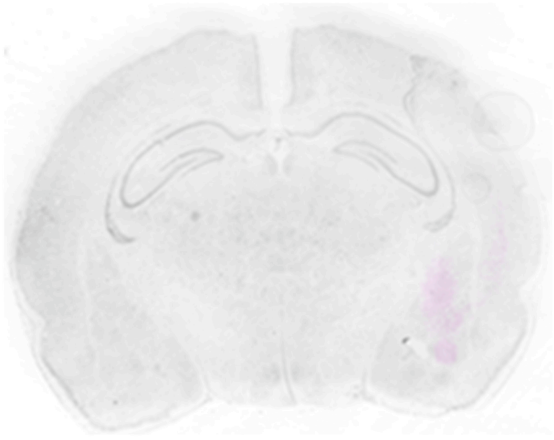
*Histological verification of cannula/probe placement and drug spread***.** Following microdialysis experiments or after testing following amygdala infusion, pups' brains were removed, frozen and sectioned at 20µm using a -20°C cryostat. Sections were stained with cresyl violet for identification of the microdialysis probes and cannula placement in relation to the amygdala using a neonatal atlas[29, 47, 62, 66] (Supplementary Figure 3).

Additional pups were implanted to characterize the extent of drug diffusion within and outside of the amygdala. These pups were infused with 2µl of a saline solution of [^{3}H] dopamine (1µCi/µl; NEN Research Products) during conditioning simulation, followed by brains removal, brain freezing in 2-methylbutane at -45°C, and sliced in 20 µm coronal sections in a cryostat. The slides were apposed to a tritium storage phosphor screen during 14 days (Amersham Biosciences, USA). Then, the screen was scanned at a pixel density of 50 µm (5000 dots per cm^2) with a STORM 820 Phosphor Imager (Molecular Dynamics, Sunnyvale, CA). Phosphorimaging of the slides results in a TIFF image file for analysis of ^{3}H diffusion[29, 47] (Supplementary Figure 3).



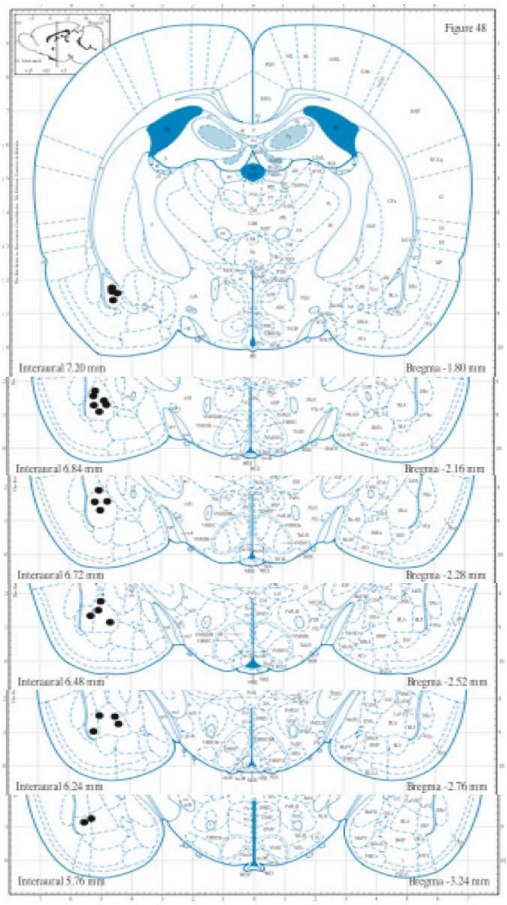
SUPPLEMENTARY FIGURES 1 AND 2. Measurements of amygdala DA metabolites, DOPAC (1a and 1b) and HVA (2a and 2b). **Figure 1.** Measurements of extracellular DOPAC efflux within the amygdala before (baseline), during (conditioning) and after (recovery) conditioning show that paired odor-shock treatment, which normally produces an odor preference in 8-day old pups, is associated with a decrease in amygdala dopamine **(a)**; in 8-day old animals that normally learn an aversion after injection with corticosterone prior to conditioning is also associated with an decrease in amygdala DOPAC **(b)**. **Figures 2 a & b**. The results for HVA were similar to those for DOPAC. ANOVA for DOPAC without CORT: significant interaction between conditioning groups X time; [$F(18,135)=55.58$, $p<0.0001$]; with CORT: significant interaction between conditioning groups X time; [$F(18,135)=54.13$, $p<0.0001$]. ANOVA for HVA without CORT: significant interaction between conditioning groups X time; [$F(18,117)=167.74$, $p<0.0001$]; with CORT: significant interaction between conditioning groups X time; [$F(18,135)=503.69$, $p<0.0001$].





SUPPLEMENTARY FIGURE 3. Injection cannula placements**.** TOP: Locations of cannula tips (solid circles) in rats used for dopamine antagonist infusion into the amygdala. Sections are from Paxinos et al. atlas[65]. BOTTOM: Color overlay of [^{3}H] dopamine diffusion within the amygdala (Storm Image) on a histological section counterstained with cresyl violet.

Microdialysis probe position



SUPPLEMENTARY FIGURE 4. Cannula placements for microdialysis. Sites were determined as described in the text. Sections are from Paxinos et al. atlas[65]. Each black circle is a cannula placement.

References

1. Pedersen, P.E. & Blass, E.M. Prenatal and postnatal determinants of the 1st suckling episode in albino rats. *Dev. Psychobiol.* **15**, 349-355 (1982).
2. Raineki, C., Moriceau, S. & Sullivan, R.M. Developing a neurobehavioral animal model of infant attachment to an abusive caregiver. (in prep).
3. Emerich, D.F., Scalzo, F.M., Enters, E.K., Spear, N.E. & Spear, L.P. Effects of 6-hydroxydopamine-induced catecholamine depletion on shock-precipitated wall climbing of infant rat pups. *Dev. Psychobiol.* **18**, 215-227 (1985).
4. Stehouwer, D.J. & Campbell, B.A. Habituation of the forelimb-withdrawal response in neonatal rats. *J. Exp. Psychol.: An. Behav. Proc.* **4**, 104-119 (1978).
5. Barr, G.A. Ontogeny of nociception and antinociception. *NIDA Res. Mono.* **158**, 172-201 (1995).
6. Fitzgerald, M. The development of nociceptive circuits. *Nature Rev. Neurosci.* **6**, 507-520 (2005).
7. Collier, A.C. & Bolles, R.C. The ontogenesis of defensive reactions to shock in preweanling rats. *Dev. Psychobiol.* **13**, 141-150 (1980).
8. Shair, H.N., Masmela, J.R., Brunelli, S.A. & Hofer, M.A. Potentiation and inhibition of ultrasonic vocalization of rat pups: regulation by social cues. *Dev. Psychobiol.* **30**, 195-200 (1997).
9. Roth, T.L. & Sullivan, R.M. Memory of early maltreatment: Neonatal behavioral and neural correlates of maternal maltreatment within the context of classical conditioning. *Biol. Psychiatry* **57**, 823-831 (2005).
10. Blozovski, D. & Cudennec, A. Passive avoidence learning in the young rat. *Dev. Psychobiol.* **13**, 513-518 (1980).
11. Collier, A.C. & Mast, J. Alleviation of avoidance deficits by approach alternatives in 10-day old rats. *Physiol. Behav.* **23**, 615-618 (1979).
12. Spear, N.E. & Rudy, J.W. Tests of the ontogeny of learning and memory: Issues, methods and results. in *Developmental psychobiology: New methods and changing concepts* (ed. H.N. Shair & G.A. Barr) 84-113 (Oxford University Press, New York, 1991).
13. Rush, A.N., Robinette, B.L. & Stanton, M.E. Ontogenetic differences in the effects of unpaired stimulus preexposure on eyeblink conditioning in the rat. *Dev. Psychobiol.* **39**, 8-18 (2001).
14. Rescorla, R.A. Pavlovian conditioning and its proper control procedures. *Psychol. Rev.* **74**, 71-80 (1967).
15. Rescorla, R.A. Behavioral studies of Pavlovian conditioning. *Annu. Rev. Neurosci.* **11**, 329-352 (1988).
16. Hoffmann, H., Hunt, P. & Spear, N.E. Ontogenetic differences in the association of gustatory and tactile cues with lithium chloride and footshock. *Behav. Neural Biol.* **53**, 441-450 (1990).
17. Campbell, B.A. & Spear, N.E. Ontogeny of memory. *Psychol. Rev.* **79**, 215-236 (1972).
18. Stanton, M.E. Multiple memory systems, development and conditioning. *Behav. Brain Res.* **110**, 25-37 (2000).
19. Bowlby, J. *Attachment* (Basic Books, New York, 1965).
20. Salzen, E.A. Imprinting and environmental learning. in *Development and Evolution of Behavior* (ed. L.R. Aronson, E. Tobach, D.S. Lehrman & J. Rosensbaltt) 158-178 (W H Freeman, San Francisco, 1970).
21. Stanley, W.E. Differential human handling as reinforcing events and as treatments influencing later social behavior in basenji puppies. *Psychol. Rep.* **10**, 775-788 (1962).
22. Harlow, H. & Harlow, M. The affectional system. in *Behavior of nonhuman primates* (ed. A. Schrier, H. Harlow & F. Stollnitz) (Academic Press, New York, 1965).

23. Maestripieri, D., Tomaszycki, M. & Carroll, K.A. Consistency and change in the behavior of rhesus macaque abusive mothers with successive infants. *Dev. Psychobiol.* **34**, 29-35 (1999).
24. Sanchez, M.M., Ladd, C.O. & Plotsky, P.M. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Dev. Psychopathol.* **13**, 419-449 (2001).
25. Suomi, S.J. Gene-environment interactions and the neurobiology of social conflict. *Ann. N Y Acad. Sci.* **1008**, 132-139 (2003).
26. Nemeroff, C.B. Neurobiological consequences of childhood trauma. *J. Clin. Psychiatry* **65 Suppl 1**, 18-28 (2004).
27. Helfer, M.E., Kempe, R.S. & Krugman, R.D. *The battered child* (University of Chicago Press, Chicago, 1997).
28. Haroutunian, V. & Campbell, B.A. Emergence of interoceptive and exteroceptive control of behavior in rats. *Science* **205**, 927-929 (1979).
29. Moriceau, S. & Sullivan, R.M. Maternal presence serves as a switch between learning fear and attraction in infancy. *Nature Neurosci.* **9**, 1004-1006 (2006).
30. Sullivan, R.M. & Leon, M. Early olfactory learning induces an enhanced olfactory bulb response in young rats. *Brain Res.* **392**, 278-282 (1986).
31. Sullivan, R.M., Landers, M., Yeaman, B. & Wilson, D.A. Good memories of bad events in infancy. *Nature* **407**, 38-39 (2000).
32. Bolles, R.C. & Woods, P.J. The ontogeny of behavior in albino rat. *An. Behav.* **12**, 427-441 (1965).
33. Blair, H.T., Schafe, G.E., Bauer, E.P., Rodrigues, S.M. & LeDoux, J.E. Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learn. Mem.* **8**, 229-242 (2001).
34. Sananes, C.B. & Campbell, B.A. Role of the central nucleus of the amygdala in olfactory heart rate conditioning. *Behav. Neurosci.* **103**, 519-525 (1989).
35. Fanselow, M.S. & LeDoux, J.E. Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* **23**, 229-232 (1999).
36. Davis, M., Walker, D.L. & Lee, Y. Roles of the amygdala and bed nucleus of the stria terminalis in fear and anxiety measured with the acoustic startle reflex. Possible relevance to PTSD. *Ann. N Y Acad. Sci.* **821**, 305-331 (1997).
37. Fanselow, M.S. & Gale, G.D. The amygdala, fear, and memory. *Ann. N Y Acad. Sci.* **985**, 125-134 (2003).
38. Herzog, C. & Otto, T. Odor-guided fear conditioning in rats: 2. Lesions of the anterior perirhinal cortex disrupt fear conditioned to the explicit conditioned stimulus but not to the training context. *Behav. Neurosci.* **111**, 1265-1272 (1997).
39. Maren, S. The amygdala, synaptic plasticity, and fear memory. *Ann. N Y Acad. Sci.* **985**, 106-113 (2003).
40. Pape, H.C. & Stork, O. Genes and mechanisms in the amygdala involved in the formation of fear memory. *Ann. N Y Acad. Sci.* **985**, 92-105 (2003).
41. Rosenkranz, J.A. & Grace, A.A. Dopamine-mediated modulation of odour-evoked amygdala potentials during pavlovian conditioning. *Nature* **417**, 282-287 (2002).
42. Schettino, L.F. & Otto, T. Patterns of Fos expression in the amygdala and ventral perirhinal cortex induced by training in an olfactory fear conditioning paradigm. *Behav. Neurosci.* **115**, 1257-1272 (2001).
43. Sevelinges, Y., Gervais, R., Messaoudi, B., Granjon, L. & Mouly, A.M. Olfactory fear conditioning induces field potential potentiation in rat olfactory cortex and amygdala. *Learn. Mem.* **11**, 761-769 (2004).
44. Sigurdsson, T., Doyere, V., Cain, C.K. & LeDoux, J.E. Long-term potentiation in the amygdala: a cellular mechanism of fear learning and memory. *Neuropharmacology* **52**, 215-227 (2007).
45. Davis, M., Walker, D.L. & Myers, K.M. Role of the amygdala in fear extinction measured with potentiated startle. *Ann. N Y Acad. Sci.* **985**, 218-232 (2003).

46. Thompson, J., Sullivan, R.M. & Wilson, D.A. Developmental emergence of fear learning corresponds with changes in amygdala synaptic plasticity. *Brain Res.* **1200**, 58-65 (2008).
47. Moriceau, S., Wilson, D.A., Levine, S. & Sullivan, R.M. Dual circuitry for odor-shock conditioning during infancy: corticosterone switches between fear and attraction via amygdala. *J. Neurosci.* **26**, 6737-6748 (2006).
48. Roozendaal, B., Carmi, O. & McGaugh, J.L. Adrenocortical suppression blocks the memory-enhancing effects of amphetamine and epinephrine. *Proc. Ntl. Acad. Sci. U S A* **93**, 1429-1433 (1996).
49. Thompson, B.L., Erickson, K., Schulkin, J. & Rosen, J.B. Corticosterone facilitates retention of contextually conditioned fear and increases CRH mRNA expression in the amygdala. *Behav. Brain Res.* **149**, 209-215 (2004).
50. Roozendaal, B. Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. *Neurobiol. Learn. Mem.* **78**, 578-595 (2002).
51. Pugh, C.R., Tremblay, D., Fleshner, M. & Rudy, J.W. A selective role for corticosterone in contextual-fear conditioning. *Behav. Neurosci.* **111**, 503-511 (1997).
52. Hui, G.K.*, et al.* Memory enhancement of classical fear conditioning by post-training injections of corticosterone in rats. *Neurobiol. Learn. Mem.* **81**, 67-74 (2004).
53. Corodimas, K.P., LeDoux, J.E., Gold, P.W. & Schulkin, J. Corticosterone potentiation of conditioned fear in rats. *Ann. N Y Acad. Sci.* **746**, 392-393 (1994).
54. Moore, C.L., Jordan, L. & Wong, L. Early olfactory experience, novelty, and choice of sexual partner by male rats. *Physiol. Behav.* **60**, 1361-1367 (1996).
55. Coopersmith, R. & Leon, M. Enhanced neural response by adult rats to odors experienced early in life. *Brain Res.* **371**, 400-403 (1986).
56. Fillion, T.J. & Blass, E.M. Infantile experience with suckling odors determines adult sexual behavior in male rats. *Science* **231**, 729-731 (1986).
57. Sevelinges, Y.*, et al.* Enduring effects of infant memories: infant odor-shock conditioning attenuates amygdala activity and adult fear conditioning. *Biol. Psychiatry* **62**, 1070-1079 (2007).
58. Sevelinges, Y., Sullivan, R.M., Messaoudi, B. & Mouly, A.M. Neonatal odor-shock conditioning alters the neural network involved in odor fear learning at adulthood. *Learn. Mem.* **15**, 649-656 (2008).
59. Hall, W.G. Feeding and behavioral activation in infant rats. *Science* **205**, 206-209 (1979).
60. Sullivan, R.M. & Wilson, D.A. Neural correlates of conditioned odor avoidance in infant rats. *Behav. Neurosci.* **105**, 307-312 (1991).
61. Breitling, R., Armengaud, P., Amtmann, A. & Herzyk, P. Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS Lett.* **573**, 83-92 (2004).
62. Shionoya, K., Moriceau, S., Bradstock, P. & Sullivan, R.M. Maternal attenuation of hypothalamic paraventricular nucleus norepinephrine switches avoidance learning to preference learning in preweanling rat pups. *Horm. Behav.* **52**, 391-400 (2007).
63. Lalumiere, R.T. & McGaugh, J.L. Memory enhancement induced by post-training intrabasolateral amygdala infusions of beta-adrenergic or muscarinic agonists requires activation of dopamine receptors: Involvement of right, but not left, basolateral amygdala. *Learn Mem* **12**, 527-532 (2005).
64. Di Ciano, P. & Everitt, B.J. Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. *J Neurosci* **24**, 7167-7173 (2004).
65. Park, W.K.*, et al.* Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *J Neurosci* **22**, 2916-2925 (2002).
66. Paxinos, G., Tork, I., Tecott, L. & Valentino, K. *Atlas of the developing rat brain* (Academic Press, San Diego, CA, 1991).