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

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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. 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, rough handling by the mother is sufficient to produce a subsequent approach to the odor.

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.
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. 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 | 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. 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. In similar work in young dogs, 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. Clinically, an analogous situation in humans includes children who form and maintain strong attachments to their abusive caregivers. 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.
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, 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. 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. Importantly, the amygdala receives information about shock and odor prior to the emergence of fear conditioning, 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, which is in sharp contrast to fear learning in adults where corticosterone only modulates learning. Indeed, it is the appearance of mature corticosterone processes, rather than amygdala development that regulates the developmental emergence of the amygdala-dependent fear learning. 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, reduce adult fear conditioning 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 \textit{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.

\textit{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.

\textit{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\textsuperscript{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\textsuperscript{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)\textsuperscript{59}.

\textit{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  |
| ProbeID      | Description                                                                 | R. norvegicus transcripted sequence with weak similarity to protein ref:NP_003903.1 (H.sapiens) myotubularin related protein 2 [Homo sapiens] | ---                         | 0.061 | -1.43 |
|--------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-----------------------------|--------|-------|
| 1389986_at   | synaptic vesicle glycoprotein 2 b                                           | Sv2b                                                                                                              | 0.062                       | 1.14   |
| 1368677_at   | brain derived neurotrophic factor                                          | Bdnf                                                                                                               | 0.062                       | -1.50  |
| 1369731_at   | membrane-associated guanylate kinase-interacting protein                   | LOC593                                                                | 0.062                       | 1.35   |
| 1388986_at   | R. norvegicus transcripted sequences                                       | ---                                                                  | 0.074                       | 1.33   |
| 1373188_at   | sodium channel, voltage-gated, type IV, beta                              | Scn4b                                                                                                               | 0.078                       | 1.59   |
| 1376278_at   | R. norvegicus transcripted sequences                                       | ---                                                                  | 0.079                       | -1.52  |
| 1368440_at   | solute carrier family 3, member 1                                          | Scl3a1                                                                | 0.081                       | 1.17   |
| 1390227_at   | R. norvegicus transcripted 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   | R. norvegicus transcripted sequence with weak similarity to protein sp:PIHUB6 (H.sapiens) PIHUB6 salivary proline-rich protein precursor PRB1 (large allele) - human | ---                                                                  | 0.083                       | 1.29   |
| 1370432_at   | POU domain, class 3, transcription factor 1                                | Pou3f1                                                                | 0.085                       | 1.29   |
| 1398245_at   | synuclein, gamma                                                           | Sncg                                                                                                               | 0.093                       | -1.56  |
| 1390722_at   | R. norvegicus transcripted sequences                                       | ---                                                                  | 0.108                       | 1.04   |
| 1369131_at   | solute carrier family 18, member 2                                         | Slc18a2                                                               | 0.117                       | -1.57  |
| 1390539_at   | R. norvegicus transcripted sequences                                       | ---                                                                  | 0.121                       | -1.09  |
| 1376967_at   | R. norvegicus transcripted sequences                                       | ---                                                                  | 0.139                       | 1.39   |
| 1390649_at   | R. norvegicus transcripted sequences                                       | ---                                                                  | 0.148                       | -1.05  |
| 1367731_at   | guanine nucleotide binding protein, beta 1                                 | Gnb1                                                                  | 0.153                       | 1.21   |
| 1373538_at   | R. norvegicus transcripted 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 | glycine transporter 1                                                      | Glyt1                                                                                                               | 0.160                       | -1.56  |
| 1383059_a at | R. norvegicus similar to testis-specific gene (LOC361202), mRNA            | ---                                                                  | 0.166                       | -1.18  |
| 1369248_a at | baculoviral IAP repeat-containing 4                                        | Birc4                                                                                                               | 0.166                       | -1.09  |
| 1387348_at   | insulin-like growth factor-binding protein 5                                | Igfbp5                                                                | 0.167                       | -1.25  |
| 1389211_at   | R. norvegicus transcripted sequences                                       | ---                                                                  | 0.168                       | 1.61   |
| 1371026_at   | protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 4 | ---                                                                  | 0.168                       | -1.30  |
| 1369297_at   | protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), gamma isoform | Ppp2r2c                                                               | 0.170                       | -1.36  |
| 1369129_at   | RAS guanyl releasing protein 1                                             | Rasgrp1                                                               | 0.172                       | 1.54   |
| 1377316_at   | R. norvegicus transcripted 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   |
| Gene ID       | Description                                                                 | Log2 Fold Change |
|--------------|------------------------------------------------------------------------------|------------------|
| 1372934_at   | R. norvegicus similar to 1700019E19Rik protein (LOC299209), mRNA            | 0.176 1.34       |
| 1371062_at   | aldehyde dehydrogenase family 5, subfamily A1                               | 0.178 1.53       |
| 1371208_at   | nucleoporin p58                                                              | 0.188 -1.11      |
| 1368708_a_at | dopamine receptor 2                                                          | 0.192 1.44       |
| 1367816_at   | global ischemia induced protein GIIG15B (LOC361188), mRNA                   | 0.199 1.49       |
| 1370333_a_at | insulin-like growth factor 1                                                 | 0.200 -1.25      |
| 1376311_at   | 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   | folate receptor 1 (adult)                                                    | 0.201 1.36       |
| 1370669_a_at | phosphodiesterase 10A                                                        | 0.208 1.37       |
| 1392592_at   | R. norvegicus transcribed sequences                                          | 0.208 -1.45      |
| 1372481_at   | R. norvegicus transcribed sequence with weak similarity to protein pir:I67604 (H.sapiens) I67604 antigen short splice form precursor - human | 0.209 1.26       |
| 1368641_at   | wingless-type MMTV integration site family, member 4                         | 0.210 -1.33      |
| 1377534_at   | 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   | echinoderm microtubule associated protein like 2                            | 0.211 1.21       |
| 1389868_at   | R. norvegicus transcribed sequences                                          | 0.211 1.31       |
| 1368312_at   | oxytocin                                                                    | 0.212 1.32       |
| 1372958_at   | R. norvegicus transcribed sequences                                          | 0.212 1.21       |
| 1371059_at   | protein kinase, cAMP-dependent, regulatory, type 2, alpha                   | 0.213 -1.29      |
| 1374065_at   | R. norvegicus transcribed sequences                                          | 0.213 1.11       |
| 1371960_at   | R. norvegicus transcribed sequences                                          | 0.213 1.24       |
| 1384101_at   | 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   | R. norvegicus transcribed sequences                                          | 0.214 -1.39      |
| 1368887_at   | cadherin 22                                                                  | 0.214 -1.24      |
| 1387450_at   | transforming growth factor alpha                                              | 0.214 1.31       |
| 1375043_at   | 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   | R. norvegicus similar to CCT (chaperonin containing TCP-1) zeta subunit (LOC303526), mRNA | 0.215 1.10       |
| Probe ID | Description                                                                 | Log2 Fold Change |
|----------|------------------------------------------------------------------------------|-----------------|
| 1374899_at | BM383043 R. norvegicus similar to hypothetical protein 4933417N17 (LOC290877), mRNA | -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) | 1.52            |
| 1375468_at | AW252983 ATP-binding cassette, sub-family C (CFTR/MRP), member 5a | -1.48           |
| 1369822_at | NM_022264 c-kit receptor tyrosine kinase | -1.32           |
| 1370751_at | U77931 calcium/calmodulin-dependent protein kinase IV | 1.19            |
| 1369753_at | M63334 R. norvegicus transcribed sequence with strong similarity to protein pir:T46507 (H.sapiens) T46507 hypothetical protein DKFZp586M2121.1 - human (fragment) | 1.07            |
| 1374879_x_at | AI228249 R. norvegicus transcribed sequences | -1.26           |
| 1376842_at | BF395964 R. norvegicus transcribed sequence with moderate similarity to protein ref:NP_077306.1 (H.sapiens) hypothetical protein MGC4365 [Homo sapiens] | 1.22            |
| 1372811_at | AA892798 uterine sensitization-associated gene 1 protein | 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 | 1.26            |
| 1387720_at | NM_134377 calystenten 2 | -1.34           |
| 1388569_at | AI179984 alpha-2 antiplasmin | -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 | -1.26           |
| 1370606_at | U22830 purinergic receptor P2Y, G-protein coupled 1 | 1.35            |
| 1388689_at | AI170755 R. norvegicus transcribed sequence with moderate similarity to protein sp:P1462 (H.sapiens) ACYM_HUMAN Acylphosphatase, muscle type isozyme (Acylphosphate phosphohydrolase) | 1.32            |
| 1374283_at | BF419505 R. norvegicus transcribed sequence with strong similarity to protein ref:NP_004500.1 (H.sapiens) fetal Alzheimer antigen [Homo sapiens] | -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) | -1.35           |
| 1369610_at | NM_021851 lin-7-C | -1.24           |
| 1376280_at | AA799789 R. norvegicus transcribed sequences | -1.29           |
| 1375648_at | BE113026 R. norvegicus transcribed sequences | 1.43            |
| 1375174_at | BI296653 R. norvegicus transcribed sequences | -1.19           |
| 1376636_at | BE111972 R. norvegicus transcribed sequences | 1.49            |
| 1399109_at | BI281673 R. norvegicus transcribed sequences | 1.33            |
| 1387541_at | NM_031653 chondroitin sulfate proteoglycan 3 | 1.10            |
| EntrezGene ID | Accession | Description                                                                                       | Value 1 | Value 2 |
|--------------|-----------|---------------------------------------------------------------------------------------------------|---------|---------|
| 1389474_at   | AA818380  | R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli)          | 0.235   | -1.18   |
| 1368957_at   | NM_024138 | guanine nucleotide binding protein, gamma 7                                                        |         |         |
| 1387274_at   | NM_012943 | distal-less homeobox 5                                                                            | 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) | 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                                                                                        | 0.239   | -1.16   |
| 1369818_at   | NM_031779 | guanine nucleotide binding protein, gamma 7                                                        |         |         |
| 137720_x_at  | AA892765  | 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] | 0.240   | 1.31    |
| 1375676_at   | AA956897  | R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli)          | 0.242   | 1.34    |
| 1387349_at   | NM_013028 | short stature homeobox 2                                                                             |         |         |
| 1368867_at   | BF281131  | GERP95                                                                                             | 0.243   | -1.38   |
| 1370770_s_at | AF071205  | Kit ligand                                                                                         | 0.243   | -1.09   |
| 1388216_at   | L03386    | Olf-1/EBF associated Zn finger protein Roaz                                                         | 0.244   | -1.26   |
| 1387170_at   | NM_053824 | casein kinase II, alpha 1 polypeptide                                                               | 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                                                                                 | 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                                                        | 0.252   | -1.28   |
| 1368263_a_at | X90402    | myelin-associated oligodendrocytic basic protein                                                   | 0.253   | -1.44   |
| 1387549_at   | NM_024361 | N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1                                          | 0.254   | -1.17   |
| 1369628_at   | BG672437  | synaptic vesicle glycoprotein 2 b                                                                  | 0.255   | -1.61   |
| Gene ID     | Description                                                                 | Fold Change |
|------------|------------------------------------------------------------------------------|-------------|
| 1387646_a_at | Max kinase interacting with leukemia-associated gene                          | 0.255       |
| 1387406_at  | R. norvegicus transcribed sequence with weak similarity to protein sp:Q9HAY2 (H.sapiens) | 0.255       |
| 1376704_a_at | R. norvegicus transcribed sequence with weak similarity to protein sp:O60664 (H.sapiens) | 0.255       |
| 1387844_at  | LIM and SH3 protein 1                                                         | 0.259       |
| 1390383_at  | R. norvegicus transcribed sequence with weak similarity to protein sp:Q9NVF9 (H.sapiens) | 0.260       |
| 1369344_at  | TGF-beta resistance-associated protein                                       | 0.260       |
| 1377921_at  | R. norvegicus transcribed sequence with strong similarity to protein sp:Q9NVF9 (H.sapiens) | 0.260       |
| 1369135_at  | Synaptotagmin 11                                                             | 0.261       |
| 1369754_a_at | Calpastatin                                                                  | 0.261       |
| 1370434_a_at | Myelin-associated oligodendrocytic basic protein                            | 0.262       |
| 1369590_a_at | DNA-damage inducible transcript 3                                            | 0.262       |
| 1374921_at  | R. norvegicus transcribed sequence with weak similarity to protein sp:Q9NVF9 (H.sapiens) | 0.262       |
| 1387341_a_at | Myelin basic protein                                                          | 0.263       |
| 1388357_at  | R. norvegicus transcribed sequence with weak similarity to protein sp:Q9UI56 (H.sapiens) | 0.263       |
| 1374276_at  | R. norvegicus transcribed sequences                                           | 0.264       |
| 1373991_at  | R. norvegicus transcribed sequence with weak similarity to protein ref:NP_079268.1 (H.sapiens) | 0.264       |
| 1388221_at  | Solute carrier family 24, member 3                                           | 0.265       |
| 1375305_at  | R. norvegicus transcribed sequence with strong similarity to protein sp:Q15531 (H.sapiens) | 0.266       |
| 1389754_at  | R. norvegicus transcribed sequence with weak similarity to protein ref:NP_477515.1 (H.sapiens) | 0.266       |
| 1374803_at  | R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) | 0.266       |
| 1376456_at  | R. norvegicus similar to nicotinamide mononucleotide adenylyltransferase 2 isoform 1; pyridine nucleotide adenyltransferase 2; chromosome 1 open reading frame 15 (LOC289095), mRNA | 0.266       |
| 1371066_at  | SNF related kinase                                                           | 0.268       |
| Accession   | Description                                                                                      | Description                                                                                      | Log2 Fold Change |
|-------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------------|
| 1375707_at | AA817993  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)                  | 0.269           |
| 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)                  | 0.269           |
| 1392246_at | AA900645  R. norvegicus transcribed sequences                                                   |                                                                                                | 0.269           |
| 1370052_at | NM_031081 3-phosphoinositide dependent protein kinase-1                                            | Pdpk1                                                                                          | 0.271           |
| 1387144_at | NM_030994 integrin alpha 1                                                                        | Itga1                                                                                          | 0.272           |
| 1375422_at | AI710284  ---                                                                                  |                                                                                                | 0.281           |
### 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 lb 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   | Ca2+/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 |
| Gene ID       | Description                                                                 | Expression Value | Fold Change |
|--------------|------------------------------------------------------------------------------|------------------|-------------|
| 1383336_at   | 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   | R. norvegicus transcribed sequences                                           | ---              | 0.198 -6.02 |
| 1371917_at   | R. norvegicus transcribed sequence with moderate similarity to protein sp:O75648 (H. sapiens) TRMU_HUMAN tRNA (5'-methylaminomethyl-2-thioridylate)-methyltransferase | ---              | 0.200 -1.48 |
| 1389211_at   | R. norvegicus transcribed sequences                                           | ---              | 0.201 -1.66 |
| 1375026_at   | R. norvegicus similar to CLN6 protein (LOC315746), mRNA                      | ---              | 0.225 -1.53 |
| 1368300_at   | adenosine A2a receptor                                                        | ---              | 0.225 -1.38 |
| 1372208_at   | 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   | R. norvegicus transcribed sequences                                           | ---              | 0.233 -1.41 |
| 1368479_at   | dopamine receptor 1A                                                          | ---              | 0.234 1.43  |
| 1388488_at   | 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 | calcitonin receptor                                                           | Calcr            | 0.260 -1.38 |
| 1387241_at   | G protein-coupled receptor 88                                                 | Gpr88            | 0.266 -1.53 |
| 1368957_at   | guanine nucleotide binding protein, gamma 7                                   | Gng7             | 0.271 -1.56 |
| 1389438_at   | R. norvegicus similar to testes development-related NYD-SP22 isoform 1 (LOC297999), mRNA | ---              | 0.271 -1.47 |
| 1370301_at   | matrix metalloproteinase 2 (72 KDa type IV collag enase)                      | Mmp2             | 0.275 -1.49 |
| 1374090_at   | R. norvegicus transcribed sequences                                           | ---              | 0.279 1.39  |
| 1374001_at   | R. norvegicus transcribed sequences                                           | ---              | 0.285 1.38  |
| 1374632_at   | R. norvegicus transcribed sequence with weak similarity to protein ref:NP_075383.1 (H. sapiens) hypothetical protein FLJ12517 [Homo sapiens] | ---              | 0.289 -1.36 |
| Gene ID       | Accession   | Description                                                                 | Enrichment | Stat1 | Stat2 |
|--------------|-------------|-----------------------------------------------------------------------------|------------|-------|-------|
| 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 |
| GenID     | 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                                  | ---    | 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 aquaporin 1 | ---    | 0.060  | 1.75 |
| 1369625_at| AA891661   | hypothetical protein RMT-7                                               | Rmt7   | 0.069  | 1.74 |
| 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     | oxytocin                                                               | 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) homeo-domain-interacting protein kinase 2 [Homo sapiens] | ---    | 0.120  | 1.42 |
| 1368802_at| NM_012625  | pro-melanin-concentrating hormone                                       | Pmch   | 0.121  | 1.81 |
| 1368730_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 |
| Gene ID      | Description                                                                 | Symbol  | Fold Change |
|-------------|------------------------------------------------------------------------------|---------|-------------|
| 1370092_at  | NM_012757 MAS1 oncogene                                                      | Mas1    | 0.145       |
| 1371442_at  | BI282904 oxygen regulated protein (150kD)                                    | Orp150  | 1.54        |
| 1374280_at  | AA817812 R. norvegicus transcribed sequences                                   | ---     | 1.51        |
| 1368751_at  | NM_031778 Shab-related delayed-rectifier K+ channel (Kv9.3)                  | Kcns3   | 1.56        |
| 1387410_at  | U72345 nuclear receptor subfamily 4, group A, member 2                        | Nr4a2   | 1.47        |
| 1390722_at  | AW531272 R. norvegicus transcribed sequences                                   | ---     | -1.35       |
| 1389734_x_at| BI282965 RT1 class lb gene(Aw2)                                                | RT1Aw2  | 1.40        |
| 1389996_at  | R. norvegicus transcribed sequences                                           | ---     | -1.76       |
| 1374958_at  | AA819640 R. norvegicus transcribed sequence with moderate similarity to protein pdb:1LBG | ---     | -1.63       |
| 1368751_at  | NM_031778 Shab-related delayed-rectifier K+ channel (Kv9.3)                  | Kcns3   | 1.56        |
| 1386941_at  | NM_022401 R. norvegicus transcribed sequence with strong similarity to protein ref:NP_000436.1 (H.sapiens) | ---     | -1.81       |
| 1369663_at  | NM_022936 cytosolic epoxide hydrolase                                         | Ephx2   | -1.69       |
| 1368479_at  | M35077 dopamine receptor 1A                                                   | ---     | 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] | ---     | -1.58       |
| 1377069_at  | BG379055 transporter-like protein                                              | Syt12   | 1.46        |
| 1376096_a_at| BE108246 R. norvegicus transcribed sequences                                   | ---     | -1.54       |
| 1368438_at  | NM_022236 phosphodiesterase 10A                                               | ---     | 1.45        |
| 1376749_at  | AA945955 R. norvegicus transcribed sequence with moderate similarity to protein pir:B35272 (H.sapiens) B35272 osteoinductive factor - human | ---     | 1.39        |
| 1370935_at  | AI105205 transporter-like protein                                              | ---     | 1.35        |
| 1388060_at  | U71294 synaptotagmin XII                                                       | LOC1713 | -1.57       |
| 1376843_at  | BE118651 R. norvegicus transcribed sequence with strong similarity to protein ref:2109341A (H.sapiens) 2109341A Ser/Thr kinase receptor:ISOTYPE=type II [Homo sapiens] | ---     | 1.46        |
| 1376419_at  | BE116226 R. norvegicus transcribed sequences                                   | ---     | -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 | ---     | 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) | ---     | -2.15       |
| 1383205_at  | BI288833 R. norvegicus transcribed sequences                                   | ---     | 1.41        |
| 1373188_at  | AI137995 sodium channel, voltage-gated, type IV, beta                         | Ddx20   | -1.53       |
| 1377236_at  | BF405622 R. norvegicus transcribed sequences                                   | Camk4   | -1.62       |
| 1373062_at  | BM388650 sulfatase FP                                                          | ---     | 1.59        |
| 1369656_at  | M36071 phosphate cytidylyltransferase 1, choline, alpha isof orm               | Nup155  | -1.63       |
| 1369919_at  | NM_019194 thyrotroph embryonic factor                                          | Cacna1a | 1.47        |
| 1390719_at  | BI294889 R. norvegicus transcribed sequences                                   | ---     | 1.44        |
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 | ggttaaccggagccttct       | 147 (324-470)   |
| 1368601-2 | solute carrier family 6 (DAT), member 3 (Slc6a3) | ctggctttagttcctggagt | accctcgtgccaatgtat       | 161 (230-390)   |
| 1387075   | tyrosine hydroxylase                              | tgtacctttgtgtcggagac | ccagttgtacgggtcaaa      | 105 (120-224)   |
| 1387075-2 | tyrosine hydroxylase                              | ggtgctgtctctctagt | ggcatactctgtcagtctgtt   | 173 (3-175)     |
| 1387022   | aldehyde dehydrogenase, family 1, member A1       | tccagttcctttatcccaaa | gtcctctcaacaaatgagt     | 119 (266-384)   |
| 1387022-2 | aldehyde dehydrogenase, family 1, member A1       | tccagtcttttctcccaaat | gtcaagggagagagcctta     | 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

| 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-1-8-02, 8mm length, 2mm membrane, 220\(\mu\)m 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 1.5\(\mu\)l/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 2\(\mu\)l of 12.5mM perchloric acid/ 250\(\mu\)M 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, 5\(\mu\)m 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. 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. 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. 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 (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 [3H] 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 cm²) with a STORM 820 Phosphor Imager (Molecular Dynamics, Sunnyvale, CA). Phosphorimaging of the slides results in a TIFF image file for analysis of 3H diffusion (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 a 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. BOTTOM: Color overlay of [3H] dopamine diffusion within the amygdala (Storm Image) on a histological section counterstained with cresyl violet.
SUPPLEMENTARY FIGURE 4. Cannula placements for microdialysis. Sites were determined as described in the text. Sections are from Paxinos et al. atlas. Each black circle is a cannula placement.
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