

**A Critique of Tania Roth's Experiments on Rats**  
**Prepared by PETA**  
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Tania Roth's main experimental interest is in epigenetics, the study of processes that alter gene activity without changing the DNA sequence, and her stated purpose is to investigate how epigenetic factors become encoded in the brain and their functional consequences on brain plasticity and behavior. To this end, she performs psychologically distressing and painful experiments in rats, despite the significant differences between rats and humans. Roth's fetal alcohol experiments, where she force feeds alcohol to neonatal rats, lack relevance to human clinical applications and use a scenario that is unrealistic for human infants. Her experiments are based on deliberate cruelty and deprivation and cause high levels of suffering in animals.

Her experiments fail to adhere to the principles of humane guidance, including the *Guide for the Care and Use of Laboratory Animals* (the Guide),<sup>1</sup> which is used by the National Institutes of Health Office of Laboratory Animal Welfare (OLAW) to direct institutes with Public Health Service (PHS) certification on humane treatment of animals. Contrary to these standards, Roth repeatedly fails to document methods of anesthesia, analgesia, and proper euthanasia in her published papers. Her publications have described frequent technical difficulties that have resulted in questionable data and increased use of animals, which violates the principles of the 3R's in research—that of replacement, reduction, and refinement.<sup>2</sup>

**Failure to adhere to the Guide and document anesthesia, analgesia, and euthanasia**

The Guide states “proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative.” The Guide specifies the use of pre-emptive and post-operative analgesia for painful procedures. In her published studies, Roth fails to describe provision of analgesia for painful procedures, including failing to note the administration of pain medication after a surgical procedure.<sup>3</sup>

The Guide also states that euthanasia methods should be consistent with the *American Veterinary Medical Association Guidelines on Euthanasia* (AVMA Guidelines)<sup>4</sup> to ensure humane killing by inducing rapid consciousness and death. In her publications, Roth omits and insufficiently describes methods of euthanasia, including decapitation of rats, which requires specialized training and demonstration of skills to perform humanely.<sup>5</sup> In her publication entitled “DNA methylation regulates neurophysiological spatial representation in memory formation,” Roth

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<sup>1</sup> National Research Council. (2011). *Guide for the care and use of laboratory animals*. Washington, D.C.: National Academy Press.

<sup>2</sup> National Centre for the Replacement, Refinement, and Reduction of Animals in Research. “The 3Rs.” [www.nc3rs.org.uk](http://www.nc3rs.org.uk).

<sup>3</sup> Roth ED, Roth TL, Money KM, SenGupta S, Eason DE, Sweatt JD. (2015). DNA methylation regulates neurophysiological spatial representation in memory formation. *Neuroepigenetics* 2:1-8.

<sup>4</sup> Leary D, et al. (2013). *AVMA Guidelines for the euthanasia of animals*. Schaumburg, IL: American Veterinary Medical Association.

<sup>5</sup> Boschen KE, Criss KJ, Palamarchouk V, Roth TL, Klintsova AY. (2015). Effects of developmental alcohol exposure vs. intubation stress on BDNF and TrkB expression in the hippocampus and frontal cortex of neonatal rats. *International Journal of Developmental Neuroscience* 43:16-24.

describes the method of euthanasia as “transcardial perfusion of formalin” without mention of other anesthetics or drugs.<sup>6</sup> Per the AVMA Guidelines, the injection of formalin is not permitted as a sole means of euthanasia, and were this the sole method used, the rats would have been subjected to painful and unacceptably cruel deaths.

The accurate and complete reporting of euthanasia methods and drugs administered, including anesthetics and analgesia, is important for transparency in scientific experiments involving animals. The ARRIVE guidelines were developed by researchers with the National Centre for the Replacement, Refinement and Reduction of Animals in Research in consultation with the scientific community to address the widespread lack of reproducibility in biomedical experiments, which empirical observations place at 75-90%.<sup>7</sup> In an effort to correct for this, the ARRIVE guidelines recommend more complete reporting on procedures and husbandry of the animals subjected to experimentation. They state that publications should “For each experiment and each experimental group, including controls, provide precise details of all procedures carried out.” This includes analgesia used, drug dosage, site and route of administration, and monitoring.<sup>8</sup> Incomplete reporting undermines the integrity of research publications, and failing to describe methods, including addressing pain and discomfort, affects the scientific and ethical validity of a publication. In every one of her publications, Roth fails to adhere to basic requirements of humane standards and ethical reporting by omitting anesthetics, pain management, and acceptable euthanasia methods.

### **Poor experimental design and frequent technical difficulties**

Tania Roth’s experiments are scientifically meaningless, making the data not only inapplicable to humans, but to rats as well (even the rats in her own studies). In several experiments,<sup>9,19</sup> Roth attempts to analyze the levels of certain genes, or markers on genes, in a brain region called the amygdala. The amygdala has numerous sub-regions that have different neuronal compositions and perform different, and in some case opposite, functions.<sup>10</sup> Because the brains of rat pups are so small, and because having larger amounts of tissue makes molecular analysis easier, Roth muddles these distinct sub-regions of the amygdala together and performs her analysis on the jumbled mixture, saying which genes went up and which went down, and then interprets what this might mean for rat behavior or development. In other words, she mixes areas of the brain that should not be compared or interchanged and makes generalizations based on this.

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<sup>6</sup> Roth ED, Roth TL, Money KM, SenGupta S, Eason DE, Sweatt JD. (2015). DNA methylation regulates neurophysiological spatial representation in memory formation. *Neuroepigenetics* 2:1-8.

<sup>7</sup> Begley CG, Ioannidis J. (2015). Reproducibility in science: improving the standard for basic and preclinical research. *Circ Res.* 116:116-126.

<sup>8</sup> Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. (2010). Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol.* 8(6): e1000412. doi:10.1371/journal.pbio.1000412

<sup>9</sup> Hill K, Warren M, Roth TL. (2014). The influence of infant–caregiver experiences on amygdala Bdnf, OXTr, and NPY expression in developing and adult male and female rats. *Behavioural Brain Research*, 272, 175-180.

<sup>10</sup> Duvarci S, Pare D. (2014). Amygdala microcircuits controlling learned fear. *Neuron* 82:966-980.

Roth commits a similar offense when she analyzes the “global methylation” of DNA in the brains of rats exposed to her various abuses.<sup>19</sup> Methylation of DNA is one of the mechanisms that the cells of body use to help control which genes are turned on or off. DNA methylation is highly specific—each gene could have different degrees of methylation at a number of different points along the gene, and methylation of the same gene varies between cells, brain regions, and other organs of the body. But again, Roth scrambles up the whole brains of rats and then says whether or not the overall, or “global,” methylation of DNA went up or down. Because DNA methylation is so specific,<sup>11</sup> this has no discernable meaning for the rats in Roth’s experiments.

In her papers, Roth describes other questionable practices, such as combining unrelated and unbalanced data just to, “enhance statistical power,”<sup>19</sup> or using inappropriate statistical analyses<sup>9</sup> that increase the chances of a false positive. For example, in data sets containing more than two experimental groups, Roth has used t-tests to compare two groups within the larger set.<sup>9</sup> By running multiple t-tests instead of a statistical test designed for multiple comparisons, Roth increases the chances of a Type I error (false positive), meaning she may have incorrectly indicated that her abuses have certain significant effects on the rats when there is no genuine effect. For data sets containing more than two groups, significance could be calculated using post-hoc tests following the two-way ANOVA, for example. In at least one published paper,<sup>9</sup> Roth plots multiple t-test comparisons on one graph and excludes control group variance from her plots, which is an incomplete and inappropriate representation of the data.

In her fetal alcohol experiments, poor experimental design and lack of relevance to human clinical conditions results in wasted resources and animal lives, which runs contrary to the principles of the 3Rs. Roth force-feeds alcohol to newborn rats via intragastric intubation, a method which in no way approximates actual human situations in which a human neonate absorbs alcohol from the mother. Roth’s painful and irrelevant methods caused high rates of neonate death in these experiments.<sup>5,12</sup> Rat pups in the control group who were sham intubated experienced such stress from the procedure that changes to *bdnf* DNA methylation in their brains were the same upon dissection as the alcohol fed pups.<sup>5</sup> This is a significant confounding factor, and calls into question any conclusions drawn from this experiment regarding fetal alcohol syndrome.

In her publication, “Changes in dam and pup behavior following repeated postnatal exposure to a predator odor (TMT): A preliminary investigation in Long-Evans rats,” technical difficulties resulted in an entire body of unusable data,<sup>13</sup> yet the experiment was published regardless of the data gaps. In the fetal alcohol experiment entitled “Effects of developmental alcohol exposure vs.

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<sup>11</sup> In Nelson HH, Marsit CJ, Kelsey KT. (2011). Global methylation in exposure biology and translational medicine. *Environ Health Perspect* 119(11):1528-1433, the authors note “Yet ‘global methylation’ is a vague term, used to interchangeably refer to various measures of cytosine methylation of the genome overall, of repetitive elements, or of multiple gene regions. In fact, these are potentially noncomparable measures arising from distinct cellular processes; therefore, greater clarity is called for when examining and reporting these disparate measures.”

<sup>12</sup> Boschen KE, McKeown SE, Roth TL, and Klintsova AY. (2016). Impact of exercise and a complex environment on hippocampal dendritic morphology, *Bdnf* gene expression, and DNA methylation in male rat pups neonatally exposed to alcohol. *Developmental Neurobiology* 77(6): 708-725.

<sup>13</sup> Ayers LW, Asok A, Blaze J, Roth TL, Rosen JB. (2016). Changes in dam and pup behavior following repeated postnatal exposure to a predator odor (TMT): A preliminary investigation in Long-Evans rats. *Developmental Psychobiology* 58:176-184.

intubation stress on BDNF and TrkB expression in the hippocampus and frontal cortex of neonatal rats,” Roth shows a similar disregard for the 3Rs when the TrkB ELISA kit failed and additional animals were added to the protocol as a result.<sup>5</sup>

Further, there is a profound issue with reproducibility in this field of epigenetic and stress-related brain changes as a result of neonatal trauma. Many publications don’t produce compatible results, and there is wide variability in findings, study design, and measured outcomes. Without any consistency in the literature, creating any meaningful body of knowledge from these studies is impossible.

### **Pain and suffering in Roth’s experiments**

Many of Roth’s experiments are predicated on inducing pain and fear in pregnant and neonatal rats. She has performed the following:

- Force-feeding alcohol to neonatal rats, resulting in the deaths of six and nine rat pups, respectively<sup>5,12</sup>
- Repeatedly shocking the feet of adult, adolescent, and neonatal rats to induce fear<sup>14</sup>
- Exposing rats to predator odor and manipulating their hormone levels to make them more fearful<sup>15</sup>
- Confining pregnant rats in tiny tubes and exposing them to strobe lights and white noise intended to distress and frighten them<sup>16</sup>
- Removing neonatal rats from their mothers and placing them with foster mothers who will mistreat them<sup>17</sup>
- Subjecting foster mothers to inadequate nesting materials intended to stress them and cause them to abuse rat pups<sup>18</sup>
- Abused rat pups are stepped on, dropped, dragged and ignored by foster mothers, and express their distress by increased audible and ultrasonic vocalizations<sup>19</sup>

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<sup>14</sup> Doherty T, Blaze J, Keller S, Roth T. (2017). Phenotypic outcomes in adolescence and adulthood in the scarcity-adversity model of low nesting resources outside the home cage. *Developmental Psychobiology*, 59(6), 703-714.

<sup>15</sup> Moriceau S, Roth T, Okotoghaide T, Sullivan R. 2004. Corticosterone controls the developmental emergence of fear and amygdala function to predator odors in infant rat pups. *International Journal of Developmental Neuroscience*, 22(5), 415-422.

<sup>16</sup> Blaze J, Asok A, Borrelli K, Tulbert C, Bollinger J, Ronca A, Roth T. (2017). Intrauterine exposure to maternal stress alters Bdnf IV DNA methylation and telomere length in the brain of adult rat offspring. *International Journal of Developmental Neuroscience*, International Journal of Developmental Neuroscience.

<sup>17</sup> Blaze J, Roth T. (2017). Caregiver maltreatment causes altered neuronal DNA methylation in female rodents. *Dev. Psychopathol.* 29(2): 477-489.

<sup>18</sup> Asok A, Bernard K, Rosen JB, Dozier M, Roth TL. (2014). Infant-caregiver experiences alter telomere length in the brain. *PLOS One* 9:e101437.

<sup>19</sup> Doherty TS, Forster A, and Roth TL. (2016). Global and gene-specific DNA methylation alterations in the adolescent amygdala and hippocampus in an animal model of caregiver maltreatment. *Behavioral Brain Research* 298:55-61.

## **Current Studies: Issues with animal welfare and lack of scientific relevance**

PETA received documents through a Freedom of Information request to the National Institutes of Health that outlined Roth's ongoing and planned experiments. These protocols document serious animal welfare concerns, including exposing female rats to abuse as babies, then breeding them and forcing them to undergo invasive surgery just one day after giving birth. This raises concerns about confounding factors of stress and immune suppression when the rats are not given adequate time to recover from parturition, as well as pain from the surgery and the stress of having to nurse and care for offspring with these additional challenges.

The experimental groups of females have drugs infused daily into their brains, then they will be killed just seven days after giving birth. One of the stated aims of Roth's current experiments is to investigate the effects of delivering a drug prior to episodes of abuse, to see if it improves maternal care in the future for the mistreated individual. She administers chromatin-modifying drugs to neonatal rats daily just prior to subjecting them to an abusive caregiver for 30 minutes. She then breeds them as adults and observes whether this affects their maternal behavior. This scenario lacks any translatability to the human context. If a human child was known to be going into an abusive situation or experiencing maltreatment, common sense dictates that rather than giving that child a drug to stave off future damage from the abuse, the abuse would be prevented or the child would otherwise be protected.

## **Conclusion**

Roth's experiments waste the lives of animals and squander resources that could be better spent on human-relevant, clinically applicable interventions. Huge populations of people suffer daily from mental health issues and the consequences of child abuse, and we are not going to find answers for them in experiments on animals. Rather than engage in cruel manipulations of rats, scientists must apply rigorous, ethical thinking to address real-world mental health needs.