Effects of Propofol and Surgical Trauma on Neurodevelopment and Cognitive Function in Developing Rats: Postprint

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Abstract

Objective: To investigate the effects of propofol and surgical trauma on neurodevelopment and cognitive function in developing rats and the underlying mechanisms. Methods: One hundred four 13-day-old SD rats were randomly divided into four groups: control, propofol, surgery, and propofol + surgery. The control group received an intraperitoneal injection of 7.5 mL/kg normal saline followed by sham surgery; the propofol group received an intraperitoneal injection of 75 mg/kg propofol; the surgery group underwent exploratory laparotomy under local anesthesia; and the propofol + surgery group received an intraperitoneal injection of 75 mg/kg propofol plus exploratory laparotomy under local anesthesia. Postoperatively, each group was randomly divided into two subgroups. One subgroup was used to detect hippocampal TNF- concentration and brain tissue expression of caspase-3 and c-fos at 1 day postoperatively. The other subgroup was raised until 60 days of age and then subjected to Morris water maze testing to evaluate cognitive function, followed by detection of hippocampal TNF- concentration and brain tissue expression of caspase-3 and c-fos. Results: In 13-day-old rats, the surgery group exhibited statistically significant differences in TNF- concentration and expression of caspase-3 and c-fos compared with the other three groups ($P<0.05$). No statistically significant differences in TNF- concentration and expression of caspase-3 and c-fos were observed among the control, propofol, and propofol + surgery groups ($P>0.05$). In 60-day-old rats, no statistically significant differences among groups were found in Morris water maze performance, TNF- concentration, or expression of caspase-3 and c-fos ($P>0.05$). Conclusion: Surgical trauma under local anesthesia can exacerbate hippocampal inflammatory response and increase apoptosis in juvenile rats, but this injury does not persist into adulthood. Single propofol general anesthesia has no significant effect on brain neuronal development

in juvenile rats, and propofol can alleviate the central inflammatory response caused by surgical trauma in young rats.

Full Text

Effect of Propofol and Operative Trauma on Neurodevelopment and Cognitive Function of the Developing Brain in Rats

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Abstract

Objective: To investigate the effect of propofol and operative trauma on the neurodevelopment and cognitive function of the developing brain and its underlying mechanisms.

Methods: A total of 104 postnatal day 13 Sprague-Dawley rats were randomly divided into 4 groups: control group (treated with 7.5 mL/kg saline and sham surgery), propofol group (treated with 75 mg/kg propofol), surgery group (undergoing abdominal surgery under local anesthesia), and propofol + surgery group (undergoing abdominal surgery under local anesthesia plus 75 mg/kg propofol). After the intervention, each group was randomly divided into two subgroups. One subgroup was used to detect hippocampal TNF- concentration and brain tissue caspase-3 and c-fos expression at 1 day postoperatively. The other subgroup was raised until 60 days of age for Morris water maze assessment of cognitive function, followed by measurement of hippocampal TNF- concentration and brain tissue caspase-3 and c-fos expression.

Results: In 13-day-old rats, the surgery group showed significantly higher TNF- concentration and caspase-3 and c-fos expression compared with the other three groups ($P<0.05$). No significant differences in these parameters were observed among the control, propofol, and propofol + surgery groups ($P>0.05$). In 60-day-old rats, no significant differences were found among the four groups in Morris water maze performance, TNF- concentration, or caspase-3 and c-fos expression ($P>0.05$).

Conclusion: Abdominal surgery under local anesthesia can induce increased inflammatory response and neuronal apoptosis in the hippocampus of neonatal rats, but this damage does not persist into adulthood. Single-dose propofol

anesthesia does not significantly affect neuronal development in young rats and can alleviate central inflammatory responses caused by surgical trauma.

Keywords: propofol; operative trauma; developing brain; cognitive function; apoptosis

Introduction

With advances in medicine, an increasing number of newborns and infants undergo surgery, invasive procedures, or imaging examinations under general anesthesia or monitored anesthesia care, which significantly improves their cooperation and comfort. Meanwhile, the effects of anesthetic agents on the developing brain have attracted growing attention. Studies have shown [1] that multiple exposures to general anesthesia before age 3 correlate with increased incidence of learning disabilities and attention deficit/hyperactivity disorder, though clinical research has yet to reach definitive conclusions [2-5].

Propofol, the most commonly used short-acting intravenous anesthetic in clinical practice, was approved by the US FDA in 1989 and recommended for clinical use, with the Netherlands and Switzerland approving its use in pediatric anesthesia in 1999. However, the effects of propofol on the developing brain remain inconclusive [6-8]. Surgical trauma can induce high levels of adrenocortical hormones and oxidative stress, leading to increased c-fos expression [9]. Therefore, we designed a two-factor study of "local anesthesia abdominal exploratory surgery"and "propofol anesthesia"to observe the short-term and long-term effects of surgical trauma and propofol anesthesia on neurodevelopment and behavior in young rats, and to explore the underlying mechanisms. This research provides a reference for rational selection of surgical timing and anesthetic agents in newborns and infants, and offers a theoretical basis for the protective role of optimized anesthesia management in perioperative neurological function in infants.

Methods

1.1 Experimental Animals

Sprague-Dawley rats, 10 days old, half male and half female, weighing 18-25 g, were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (animal license number: SCXK (Beijing) 2016-0011). The study was approved by the Ethics Committee of the General Hospital of the People' s Liberation Army. The rats were acclimated for 3 days under conditions of 20-25°C room temperature, 60-70% humidity, with fresh air and good ventilation, and were co-housed with their mothers.

1.2 Main Drugs, Reagents, and Instruments

Propofol injection (10 mg/mL, AstraZeneca, batch number: MV039); Ropivacaine hydrochloride injection (75 mg/mL, AstraZeneca, H20140763); Rat TNF-

ELISA kit (Abcam, product number: ab100785); Thermo MK3 microplate reader (Thermo, USA); 3-18K high-speed low-temperature centrifuge (Sartorius, Germany); Automatic tissue homogenizer (Roche, USA).

Experimental Models

(1) Abdominal surgery under local anesthesia: Young rats were secured with tape on a heating pad (37°C). After abdominal hair removal, disinfection, and draping, 0.1 mL of 0.5% ropivacaine was administered layer-by-layer for local anesthesia. A midline abdominal incision of approximately 2.5 cm was made to expose the abdominal cavity. Sterile forceps were used to sequentially explore the liver, spleen, stomach, small intestine, colon, and other viscera. The muscle and skin were then sutured layer-by-layer with silk thread. The surgical procedure lasted 1 hour.

(2) Sham surgery: Young rats were secured, had abdominal hair removed, and were disinfected, but no surgery was performed.

(3) Propofol anesthesia: Intraperitoneal injection of propofol injection at 75 mg/kg.

Experimental Grouping

A total of 104 13-day-old (P13) Sprague-Dawley rats were numbered by body weight and randomly divided into 4 groups (n=26 each) using CHISS software: control group (con), propofol group (ppf), surgery group (sur), and propofol + surgery group (ppf+sur). The con group received intraperitoneal injection of 7.5 mL/kg normal saline followed by sham surgery. The ppf+sur group underwent local anesthesia surgery after the righting reflex disappeared following propofol anesthesia. After model establishment, each group was randomly divided into two subgroups: one subgroup underwent detection of hippocampal TNF- and brain tissue caspase-3 and c-fos at 1 day postoperatively; the other subgroup was housed separately and raised until 60 days old (P60) for Morris water maze testing followed by detection of hippocampal TNF- and brain tissue caspase-3 and c-fos.

1.4 ELISA Detection of TNF- Content in Hippocampal Tissue

Eight rats were randomly selected from each group at 1 day after model establishment for hippocampal ELISA detection. Young rats were decapitated, brains were rapidly removed on ice, hippocampi were dissected and stored in liquid nitrogen. Forty milligrams of tissue sample were weighed, and RIPA lysis buffer, protease inhibitor, and phosphatase inhibitor PMSF were added. The samples were homogenized by shaking, lysed, and centrifuged. The supernatant was collected for ELISA detection. In the other subgroup, after behavioral testing at 60 days, 8 rats from each group were selected for hippocampal TNF- detection.

1.5 Immunohistochemical Detection of Caspase-3 and c-fos Expression in Brain Tissue

Five rats were randomly selected from each group at 1 day after model establishment. They were anesthetized with intraperitoneal injection of 10% chloral hydrate at 35 mg/kg. The chest was opened to expose the heart, a cannula was inserted through the left ventricle and connected to a perfusion system, and the right atrial appendage was cut as an outlet for perfusate. Normal saline was rapidly perfused until the outflow became clear and the liver turned uniformly pale, followed by slow perfusion with 4% paraformaldehyde for fixation. Brain tissue was removed and fixed in 4% paraformaldehyde for 24 hours. Immunohistochemistry was used to detect caspase-3 and c-fos expression. Routine paraffin embedding was performed, and consecutive 4 m thick sections were cut. Sections were deparaffinized with xylene and conventional gradient alcohol, endogenous peroxidase was blocked with 3% H2O2, antigen retrieval was performed with citrate buffer, and primary antibodies (caspase-3 antibody, c-fos antibody, dilution 1:100, purchased from Abcam, USA) were added and incubated at 4°C for 24 hours. Secondary antibody (goat anti-rabbit secondary antibody working solution, PV-9001 kit, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) was then added. Between each step, sections were washed with 0.01 mol/L PBS buffer for 5 minutes × 3 times, followed by DAB color development, dehydration, clearing, and mounting. Observation was performed under light microscopy (40×), and Image-Pro image analysis system was used for analysis. In the other subgroup, after behavioral testing at 60 days, 5 rats from each group were randomly selected for immunohistochemical detection.

1.6 Morris Water Maze (MWM) Experiment

The Morris water maze was used to assess spatial learning and memory abilities in rats. The apparatus consisted of a circular pool 1.2 m in diameter and 0.5 m deep, filled with water at 22±1°C to a depth of 30 cm, and divided into four quadrants.

Navigation training: The pool was divided into four quadrants by direction. A platform 10 cm in diameter was placed in the first quadrant, submerged 1.5 cm below the water surface. Rats were placed in the water at the midpoint of the first quadrant facing the pool wall. If a rat found the platform, the time taken to locate it (in seconds) was recorded as the "escape latency," and the rat remained on the platform for 10 seconds. If a rat failed to find the submerged platform within 60 seconds, the time was recorded as 60 seconds and the rat was placed on the platform for 10 seconds. The animal was then removed, dried, warmed under a lamp for 5 minutes, and returned to its cage. After all rats completed training in the first quadrant, training was sequentially conducted in quadrants 2-4. Each rat was trained 4 times daily for 5 consecutive days, and swimming speed was simultaneously recorded.

Spatial probe test: On day 6, the platform was removed. Rats were placed

in the quadrant opposite the platform quadrant facing the pool wall, and the "time spent in the target quadrant" and "number of crossings over the original platform location" within 60 seconds were recorded.

1.7 Statistical Analysis

SPSS 22.0 statistical software was used for analysis. Measurement data were expressed as mean ± standard deviation. Inter-group comparisons were performed using factorial design ANOVA, with LSD test for pairwise comparisons. Repeated measures ANOVA was used for Morris water maze data. $P<0.05$ was considered statistically significant.

Results

2.1 TNF- in Hippocampal Tissue and Caspase-3, c-fos Expression in 13-Day-Old Rats

TNF- content in hippocampal tissue at 1 day after model establishment (Figure 1 [Figure 1: see original paper]): Compared with the con group, TNF- content in the hippocampus was significantly increased in the sur group ($P<0.05$). In contrast, TNF- content was significantly decreased in the ppf+sur group compared with the sur group ($P<0.05$). No significant differences were observed between the ppf group, ppf+sur group, and con group ($P>0.05$).

Caspase-3 and c-fos expression results (Figure 2 [Figure 2: see original paper]): Compared with the con group, caspase-3 ($P<0.05$) and c-fos expression ($P<0.01$) in brain tissue were significantly increased in the sur group. However, compared with the sur group, caspase-3 ($P<0.05$) and c-fos expression ($P<0.01$) were significantly decreased in the ppf+sur group. No significant differences were found between the ppf group, ppf+sur group, and con group ($P>0.05$).

2.2 Morris Water Maze, Hippocampal TNF- , and Brain Caspase-3, c-fos Expression at 60 Days

Morris water maze results (Figure 3 [Figure 3: see original paper]): No significant differences in swimming speed were observed among the four groups. Escape latency during navigation training showed no significant differences among the con, sur, ppf, and ppf+sur groups. In the reference memory test, time spent in the target quadrant and number of platform crossings also showed no significant differences among groups.

TNF- ELISA detection in hippocampal tissue after Morris water maze testing: Results (Figure 4 [Figure 4: see original paper]) showed no significant differences among groups.

Immunohistochemical detection of caspase-3 and c-fos expression in

brain tissue (Figure 5 [Figure 5: see original paper]): No significant differences were observed among groups.

Figure 1. Concentration of TNF- in the hippocampus of the P13 SD rats. *P<0.05 vs con group; #P<0.05 vs sur group.

Figure 2. Expression of caspase-3 and c-fos in the brain of the P13 rats. A: caspase-3; B: c-fos. *P<0.05 vs con group; #P<0.05 vs sur group; **P<0.01 vs con group; ##P<0.01 vs sur group.

Figure 3. Morris water maze test results in the 4 groups. A: Escape latency in navigation training; B: Swimming speed; C, D: Time in target quadrant (C) and crossing times (D).

Figure 4. Concentration of TNF- in the hippocampus of the P60 SD rats.

Figure 5. Expression of caspase-3 and c-fos in the brain of the P60 rats. A: caspase-3; B: c-fos.

Discussion

The FDA recently issued a widely noticed drug safety announcement [10] stating that multiple or prolonged use of general anesthetic or sedation drugs in pregnant women or children may harm the brains of fetuses or children under 3 years of age. Consequently, the timing of surgery or anesthesia in infants has become a research focus. Many previous studies have used single or multiple propofol exposures in 7-day-old SD rats to investigate anesthetic effects on the brain [6-7,11], but age conversion between 7-day-old SD rats and humans is difficult. Our study aimed to observe the effects of propofol anesthesia and surgical trauma during the period from the neonatal stage (within 28 days after birth) to early infancy (<3 months) on the developing brain. Thirteen-day-old SD rats correspond to 41-day-old humans [12], while 60-day-old rats correspond to approximately 13-year-old humans. Additionally, pilot experiments found that 7-day-old rats could not tolerate abdominal surgery, so we selected 13-day-old SD rats as our subjects, with 60-day-old rats as the study endpoint.

Our study found that abdominal surgery under local anesthesia in 13-day-old rats increased TNF- release in the hippocampus and upregulated caspase-3 and c-fos protein expression, indicating increased hippocampal neuronal apoptosis. Previous studies have shown that inflammatory factors produced after surgical trauma can lead to cognitive decline [13-15]. The hippocampus is extremely sensitive to inflammatory responses [16], which has been confirmed in postoperative rats through tests of spatial learning and memory [17-18] and fear memory [19-21]. TNF- is an important pro-inflammatory cytokine with multiple biological effects that is released early after brain injury. C-fos is a member of the immediate early oncogene family and marks the beginning of neuronal activation and excitatory response activity. Excessive expression of both TNF- and c-fos can produce neurotoxicity [22] and accelerate cell apoptosis. Caspase-3, as an important effector enzyme in pro-apoptotic signal transduction pathways,

is the executor of apoptosis and a characteristic marker of cell death. Surgical trauma promotes increased release of inflammatory mediators in the hippocampus, causing glial cells to produce Fos protein [23] and upregulating c-fos expression, which regulates downstream target genes to reduce synthesis of cell survival proteins and increase killer proteins, thereby accelerating hippocampal neuronal apoptosis. Studies by Yao et al. [24-25] demonstrated that surgical trauma can increase hippocampal inflammatory mediator release and Fos protein expression, which positively correlates with neuronal apoptosis. These results are consistent with our findings.

Our study also found that single-dose propofol anesthesia did not increase inflammatory mediator release or caspase-3 and c-fos expression in 13-day-old SD rats, and propofol could alleviate the effects caused by surgical trauma. Previous studies have confirmed that propofol has protective effects on the brain, possibly because it can reduce cerebral metabolic rate, lower intracranial pressure, decrease release of excitatory glutamate neurotransmitters and pathological changes induced by neurotoxicity, inhibit lipid peroxidation, prevent protein denaturation, reduce inflammatory mediator release, and thereby decrease/inhibit cell apoptosis, thus exerting protective effects on the brain. Research by Li et al. [26-27] demonstrated that propofol can inhibit NF- B activation and high expression of caspase-3 and TNF- to a certain extent, preventing further expansion of the inflammatory cascade and protecting brain tissue by inhibiting neuronal apoptosis. These conclusions are consistent with our study results.

Cognitive activity is a high-level neural function of the brain and an important intellectual factor, with learning and memory as its components. Our study also confirmed that single-dose propofol anesthesia and abdominal surgery under local anesthesia in 13-day-old SD rats did not impair learning and memory function at 60 days. Anders et al. [28] also found that single injection of 60 mg/kg propofol in 10-day-old mice did not cause cognitive decline in adulthood, consistent with our results. The rapid developmental period for learning and memory in rats is the late gestational period and the first two weeks after birth, during which the immature nervous system is highly sensitive to changes in internal and external environments. Meanwhile, the first two weeks after birth is also the peak period of neurodevelopment, during which the nervous system has strong adaptive and self-repair functions [29]. With the disappearance of inflammation and apoptosis-inducing factors, activation of neural stem cells and progression of damage repair, proliferation and differentiation of endogenous neural stem cells gradually compensate for hippocampal neuronal damage, thereby restoring the neural network structure. This may explain why no cognitive or neurological function impairment was detected in any group at 60 days.

In summary, local surgery in young rats can cause increased release of brain inflammatory mediators and upregulated expression of caspase-3 and c-fos. However, the effects of such surgical trauma do not persist into adulthood. Meanwhile, single-dose propofol anesthesia does not affect the central nervous sys-

tem of young rats and can alleviate brain neuronal damage caused by surgical trauma.

References

[1] Hu D, Flick RP, Zaccariello MJ, et al. Association between Exposure of Young Children to Procedures Requiring General Anesthesia and Learning and Behavioral Outcomes in a Population-based Birth Cohort [J]. Anesthesiology, 2017, 127(2): 227-40.

[2] Lei SY, Hache M, Loepke AW. Clinical research into anesthetic neurotoxicity: does anesthesia cause neurological abnormalities in humans [J]. J Neurosurg Anesthesiol, 2014, 26(4): 349-57.

[3] Ing C, Dimaggio C, Whitehouse A, et al. Long-term differences in language and cognitive function after childhood exposure to anesthesia [J]. Pediatrics, 2012, 130(3): e476-85.

[4] Backeljauw B, Holland SK, Altaye M, et al. Cognition and brain structure following early childhood surgery with anesthesia [J]. Pediatrics, 2015, 136(1): e1-12.

[5] Sun LS, Li G, Miller TL, et al. Association between a single general anesthesia exposure before 36 months and neurocognitive outcomes in later childhood [J]. JAMA, 2016, 315(21): 2312-20.

[6] Cattano D, Young C, Straiko MM, et al. Subanesthetic doses of propofol induce neuroapoptosis in the infant mouse brain [J]. Anesth Analg, 2008, 106(6): 1712-4.

[7] Yu D, Jiang Y, Gao J, et al. Repeated exposure to propofol potentiates neuroapoptosis and long-term behavioral deficits in neonatal rats [J]. Neurosci Lett, 2013, 534(1): 41-6.

[8] Nyman Y, Fredriksson A, Lonnqvist PA, et al. Etomidate exposure in early infant mice (P10) does not induce apoptosis or affect behaviour [J]. Acta Anaesthesiol Scand, 2016, 60(5): 588-96.

[9] Huang YY, Kandel ER, Varshavsky L, et al. A genetic test of the effects of mutations in PKA on mossy fiber LTP and its relation to spatial and contextual learning [J]. Cell, 1995, 83(7): 1211-22.

[10] FDA. FDA Drug Safety Communication: FDA review results in new warnings about using general anesthetics and sedation drugs in young children and pregnant women [EB/OL]. https://www.fda.gov/Drugs/DrugSafety/ucm532356.htm, 2017-04-28/2017-11-07.

[11] Cao YL, Zhang W, Ai YQ, et al. Effect of propofol and ketamine anesthesia on cognitive function and immune function in young rats [J]. Asian Pac J Trop Med, 2014, 7(5): 407-11.

[12] Workman AD, Charvet CJ, Clancy B, et al. Modeling transformations of neurodevelopmental sequences across mammalian species [J]. J Neurosci, 2013, 33(17): 7368-83.

[13] Cao XZ, Ma H, Wang JK, et al. Postoperative cognitive deficits and neuroinflammation in the hippocampus triggered by surgical trauma exacerbated in aged rats [J]. Neuropsychopharmacol Biol Psychiatry, 2010, 34(8): 1426-32.

[14] He HJ, Wang Y, Le Y, et al. Surgery upregulates high mobility group box-1 and disrupts the blood-brain barrier causing cognitive dysfunction in aged rats [J]. CNS Neurosci Ther, 2012, 18(12): 994-1002.

[15] Rosczyk HA, Sparkman NL, Johnson RW. Neuroinflammation and cognitive function in aged mice following minor surgery [J]. Exp Gerontol, 2008, 43(9): 840-6.

[16] Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis [J]. Brain Behav Immun, 2011, 25(2): 181-213.

[17] Wuri G, Wang DX, Zhou Y, et al. Effects of surgical stress on long-term memory function in mice of different ages [J]. Acta Anaesthesiol Scand, 2011, 55(4): 474-85.

[18] Tan WF, Cao XZ, Wang JK, et al. Protective effects of lithium treatment on spatial memory deficits induced by surgery via tau hyperphosphorylation in splenectomized rats [J]. Clin Exp Pharmacol Physiol, 2010, 37(10): 1010-5.

[19] Fidalgo AR, Cibelli M, White JP, et al. Peripheral orthopaedic surgery down-regulates hippocampal brain-derived neurotrophic factor and impairs remote memory in mouse [J]. Neuroscience, 2011, 190(36): 194-9.

[20] Fidalgo AR, Cibelli M, White JP, et al. Systemic inflammation enhances surgery-induced cognitive dysfunction in mice [J]. Neurosci Lett, 2011, 498(1): 63-6.

[21] Terrando N, Monaco C, Ma D, et al. Tumor necrosis factor-alpha triggers a cytokine cascade yielding postoperative cognitive decline [J]. Proc Natl Acad Sci USA, 2010, 107(47): 20518-22.

[22] Gao YJ, Ji RR. c-Fos and pERK, which is a better marker for neuronal activation and central sensitization after noxious stimulation and tissue injury [J]? Open Pain J, 2009, 2(1): 11-7.

[23] Vareli K, Frangou-Lazaridis M. Prothymosin is localized in mitotic spindle during mitosis [J]. Biol Cell, 2004, 96(6): 421-8.

[24] Yao X, Peng M, Lu SQ, et al. Effect of surgical trauma on expression of inflammatory mediators in hippocampus of aged rats [J]. Wuhan University Journal: Medical Edition, 2013, 34(2): 171-3.

[25] Tong MF, Liu WG. Study on relationship between c-fos gene expression level and cell apoptosis at different stages after traumatic brain injury in rats

[J]. Zhejiang Journal of Traumatic Surgery, 2009, 14(5): 432-3.

[26] Li J, Han B, Ma X, et al. The effects of propofol on hippocampal caspase-3 and Bcl-2 expression following forebrain ischemia-reperfusion in rats [J]. Brain Res, 2010, 1356(3): 11-23.

[27] Ren XL, Gui JY, Li HB, et al. Expression of NF- B and TNF- in brain tissue during acute phase of traumatic brain injury in rats and intervention effect of propofol [J]. Modern Medicine and Health, 2011, 27(9): 1297-9.

[28] Fredriksson A, Ponten ET, Eriksson P. Neonatal exposure to a combination of N-methyl-D-aspartate and gamma-aminobutyric acid type A receptor anesthetic agents potentiates apoptotic neurodegeneration and persistent behavioral deficits [J]. Anesthesiology, 2007, 107(3): 427-36.

[29] Sturman DA, Moghaddam B. The Neurobiology of Adolescence: Changes in brain architecture, functional dynamics, and behavioral tendencies [J]. Neurosci Biobehav Rev, 2011, 35(8): 1704-12.

Note: Figure translations are in progress. See original paper for figures.