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Memory deficits associated with sublethal cyanide poisoning relative to cyanate toxicity in rodents

S. Kimani^{1,2}, K. Sinei¹, F. Bukachi³, D. Tshala-Katumbay⁴, and C. Maitai¹

¹Department of Pharmacology and Pharmacognosy, University of Nairobi, Kenya

²School of Nursing Sciences, University of Nairobi, Kenya

³Department of Medical Physiology, University of Nairobi, Kenya

⁴Center for Research on Occupational & Environmental Toxicology & Department of Neurology, OHSU, USA

Abstract

Background—Food (cassava) linamarin is metabolized into neurotoxicants cyanide and cyanate, metabolites of which we sought to elucidate the differential toxicity effects on memory.

Methods—Young 6-8 weeks old male rats were treated intraperitoneally with either 2.5 mg/kg body weight (bw) cyanide (NaCN), or 50 mg/kg bw cyanate (NaOCN), or 1 µl/g bw saline, daily for 6 weeks. Short-term and long-term memories were assessed using a radial arm maze (RAM) testing paradigm.

Results—Toxic exposures had an influence on short-term working memory with fewer correct arm entries ($F_{2, 19} = 4.57$, $p < 0.05$), higher working memory errors (WME) ($F_{2, 19} = 5.09$, $p < 0.05$) and longer RAM navigation time ($F_{2, 19} = 3.91$, $p < 0.05$) for NaOCN relative to NaCN and saline treatments. The long-term working memory was significantly impaired by cyanide with fewer correct arm entries ($F_{2, 19} = 7.45$, $p < 0.01$) and increased working memory errors ($F_{2, 19} = 9.35$, $p < 0.05$) in NaCN relative to NaOCN or vehicle treated animals. Reference memory was not affected by either cyanide or cyanate.

Conclusion—Our study findings provide an experimental evidence for the biological plausibility that cassava cyanogens may induce cognition deficits. Differential patterns of memory deficits may reflect the differences in toxicity mechanisms of NaOCN relative to NaCN. Cognition deficits associated with cassava cyanogenesis may reflect a dual toxicity effect of cyanide and cyanate.

Keywords

cognition; cyanide; cyanate; cassava

Introduction

Cassava is a staple food to over half a billion people. The metabolic pathway of cassava cyanogens in human as well as animals is well documented. Under normal conditions, ingested linamarin, the main cyanogenic glycoside in cassava, is converted to acetone cyanohydrin and cyanide (CN), which in turn is converted to the less acutely toxic thiocyanate (SCN). This metabolic conversion is done via a sulphur amino acid (SAA)-

dependent rhodanese pathway (Spencer 1999). CN is also converted into trace amounts of cyanate (OCN) and 2- aminothiazoline-4-carboxylic acid (ATCA) (Rosling 1994; Essars et al. 1992; Carlsson et al. 1995). Dependency on insufficiently processed cassava and low dietary intake of SAA has been implicated in the pathogenesis of Konzo (Banea-Mayambu et al. 1997; Cliff et al. 2011; Nzwalo and Cliff 2011), an upper motor neuron and paralytic disease prevalent in poor rural African communities (Rosling et al. 1988; Rosling and Tylleskar 1995). Candidates for such neurodisability include cyanide, or cyanate, sulfur deficiency, or their respective combinations (Kassa et al. 2011).

Exposure to cyanide or cyanate may also emanate from other sources namely, cassava processing (Okafor et al. 2002), industrial usage in metal cleaning and polishing (Mathangi and Namasivayan 2000), or occupational exposure from fire smoke or warfare (Amizet et al. 2011; Skyles 1981). Cyanide may pose imminent risk of acute toxicity and lethal threat if accidentally or intentionally used in chemical warfare and/or genocidal activities (Sykes 1981). In konzo-endemic areas, the major source of cyanide exposure has mainly been from consumption of insufficiently processed bitter cassava (Essers et al. 1992; Tylleskar et al. 1991). A recent study has suggested that children reliant on cyanogenic cassava as main source of food may present with subtle but pervasive cognitive deficits that have been so far overlooked. These findings raised global health concerns as cyanogenic cassava is a staple crop for millions of people around the globe (Boivin et al. 2013). Understanding the neurotoxicity of cyanogenic cassava has been difficult due to the lack of an experimental model. In this study, we sought to determine whether cognition notably memory functions are impaired by sublethal cyanide poisoning or cyanate toxicity, both at the pathogenetic roots of cassava-associated neurodisability.

2. Materials and methods

2.1. Chemicals

NaCN (CAS No. 143-33-9, 97.2% purity) and NaOCN (CAS No. 917-61-3, 96% purity) were bought from Sigma-Aldrich (St. Louis, MO) and stored at room temperature. All other laboratory reagents were of analytical or molecular biology grades.

2.2. Animals

Young adult Sprague-Dawley male white albino rats, 6-8 weeks old (N = 22), weighing 120-230 g upon arrival were used. The rats were acquired from the Department of Zoology, School of biological sciences, University of Nairobi, which maintains animals for experimental studies. These are the commonly breed and used strain of rats in the laboratory and have also been extensively used in neurobiological research (Kuramoto et al. 2012). The animals were caged in an experimental room in toxicology laboratory at the department of Pharmacology and Pharmacognosy, University of Nairobi. The rats were used as per institutional regulations on colony handling and efficient use of laboratory animals.

2.3. Diet

A normal rodent pellet diet was purchased from Unga Feeds, (Nairobi, Kenya). This is a commercially available balanced diet for rats with necessary vitamins and minerals. Animals were fed and given tap water *ad libitum* for 6 weeks during acclimatization and treatment phases. During both short and long-term memory testing, rats were fed on 85% food and water *ad libitum*. The 85% food restriction was based on the fact that when rodents are partially starved, they get motivated to explore and search for food in the arms of the RAM therefore encouraging navigation (Hodges 1996).

2.4. Dosing regimens

Rats were acclimated for a 5-day period on a diet consisting of normal rodent pellet, Unga Feeds (Nairobi, Kenya). On day 6, animals were assigned to experimental groups (N = 7-8/group) and treated intraperitoneally (i.p) (one injection per day) for 6 weeks as follows: (1) 2.5 mg/kg body weight (bw) NaCN; (2) 50 mg/kg bw NaOCN; (3) equivalent amount of vehicle (1 µl/g bw saline). The rats were weighed daily to assess changes in body weight and to adjust the dose of the test articles.

2.5. Animal observations

Rodents were examined daily for physical signs of disease including tremors and the hind limb extension reflex which is elicited by gently raising the animal by the tail.

2.6. Radial arm maze assessment

The assessment was based on performance of rats on custom-made wooden radial arm maze (RAM), a paradigm that has been used for testing spatial working and reference memory (Olton and Samuelson 1976; Olton 1985; Wenk et al. 1986). The assessment was carried out in two phases, namely; working (short-term) memory (defined as information that is useful to the rat during the current experience with the task) and reference (long-term) memory (information that is useful in all exposures to the task across all the days of testing) (Olton 1985; Wenk et al. 1986). Short-term (working) memory was tested for 8 consecutive days with one trial per day that was preceded by 3 days of habituation (15 min trial per day). The reference (long-term) memory was assessed thereafter for 5 days with two trials per day.

During the testing, rats were individually placed on the RAM made of 8 arms measuring 50 cm length, 10 cm width and 5 cm height. The RAM had a central octagonal platform measuring 25 cm with eight arms extending from it like the spokes of a wheel. RAM was fitted with guillotine doors that were opened and closed individually; it also separated the central platform from the arms. The arms had drilled holes where food pellets were kept to motivate the animals. The maze was raised one meter above the floor of the room that was enriched with cartoons to serve as spatial cues. The investigator assumed the same position throughout the experimentation. Rodent urine and feces were cleaned immediately after each animal trial to minimize intra-maze cues that may influence cognitive performance.

During long-term memory testing, rats were allowed 60 seconds after the first trial for the commencement of the second trial. The RAM assessment parameters namely; correct entry counts into unvisited arms, re-entry counts into visited arms (working memory errors (WME) and RAM navigation time were used to assess working memory (short-term memory) during each trial (10 minutes) for 8 days. During reference (long-term) memory assessment, only four out of the eight arms were baited. The following parameters were measured; correct entry counts into baited arms, entry counts into un-baited arms (reference memory errors (RME), re-entry counts into baited arms (WME) and RAM navigation time.

2.3. Statistical analysis

Rat body weights were analyzed by ANOVA to determine the effect of treatment on the different parameters of weight. The mean bodyweight changes across the three treatments were analyzed in two stages. A simple linear regression to estimate the initial body weight and mean daily weight change for each animal over the 6 weeks. An ANOVA followed by Bonferroni multiple comparison post hoc tests was used to determine whether the two parameters were affected by treatment. The RAM parameters namely; correct arm entry counts, re-entry counts (WME), entry counts to the unbaited arm (RME), RAM navigation time during working and reference memory were analyzed by ANOVA followed by Bonferroni post hoc tests to establish between and within group differences in terms of the

mean and changes of the parameters with each trial over the testing period. All the analyses were conducted at the significance level of $p < 0.05$.

3. RESULTS

3.1. Physical and behavioral observations

The experimental animals did not show any overt physical signs of motor deficit. Except for mild restlessness among the rats treated with cyanide, other signs of acute toxicity namely seizures, breathing problems and restlessness within the initial and subsequent phases of experimentation were not observed with cyanide or cyanate.

3.2. Body weight changes

Rats in all the treatment groups gained weight over days albeit differentially (Fig. I). The change in weight was significantly ($F_{2, 19} = 4.11$, $p < 0.05$) influenced by treatment. The mean weight change per day over the experimental period was, NaCN (2.73 ± 0.6), NaOCN (2.28 ± 0.5) relative to controls (3.11 ± 0.5) g. Post hoc test revealed that treatment with NaOCN resulted in significantly ($p < 0.05$) slower rate in weight gain relative to control rats. The change in weight for NaOCN compared to NaCN treated rats was not statistically different ($p = 0.59$).

3.3. Short-term memory

The short-term memory results in rats were summarized in terms of number of correct arm entry counts made into all the 8 baited arms, re-entry counts into already visited arms (WME) and the RAM navigation time. There was significant effect ($F_{2, 19} = 4.57$, $p < 0.05$) of treatment on correct arm entry counts into unvisited arm by rats navigating the RAM (Fig. IIa). The mean correct arm entry counts across the trials among rats treated with NaOCN (3.6 ± 0.7) were fewer relative to NaCN (4.1 ± 0.4) and controls (6.2 ± 0.6). The correct arm entry counts decreased significantly ($p < 0.05$) with each trial in NaOCN (-0.19 ± 0.2), relative to NaCN (0.13 ± 0.2) and saline (0.16 ± 0.3) treated rats. However, no statistical difference ($p = 0.8$) in correct arm entry counts was found with each trial between NaCN compared to the saline treated rats over the testing period.

The re-entry counts into already visited arms (WME) were significantly influenced ($F_{2, 19} = 5.09$, $p < 0.05$) by treatment during RAM testing (Fig. IIb). The mean re-entry counts across the trials in rats treated with NaOCN (85.05 ± 7.7) were higher ($p < 0.05$) relative to NaCN (77.98 ± 4.4) and saline (26.11 ± 2.6) %, respectively. The re-entries increased with each trial in NaOCN (8.40 ± 2.2) compared to a decrease in NaCN (-1.18 ± 0.4) ($p = 0.05$) and control (-3.21 ± 0.9) ($p = 0.01$) groups. However, no statistical ($p = 0.5$) difference was found with each trial between NaCN and saline treated rats on re-entry counts over the testing period.

The RAM navigation time was significantly ($F_{2, 19} = 3.91$, $p < 0.05$) influenced by treatment among experimental rats (Fig. IIc). The mean RAM navigation time across the trials in rats treated with NaOCN, NaCN relative to controls during short-term memory testing were (6.26 ± 0.3), (4.72 ± 0.1) and (2.82 ± 0.3) minutes. The navigation time changed with each trial, it increased in NaOCN (0.25 ± 0.03) and NaCN (0.02 ± 0.01) relative to saline (-0.01 ± 0.02) treated rats. The change in navigation time with each trial was also significantly ($p < 0.05$) higher in the rats treated with NaOCN compared to controls. However, comparing the change between NaOCN relative to NaCN treated rats with each trial, no statistical difference ($p = 0.23$) was yielded over the testing period.

3.4. Long-term memory

The long-term memory performance results were summarized in terms of number of correct arm entry counts into baited arms, re-entry counts into already visited baited arms (WME), entry counts into un-baited arms (RME) and the RAM navigation time with only 4 out of 8 baited arms. There was significant effect ($F_{2, 19} = 7.45$ $p < 0.01$) of treatment on the correct arm entry counts into the baited arms (Fig. IIIa). The mean arm entry counts across the 10 trials among rats treated with NaOCN, NaCN relative to saline were (3.1 ± 0.6), (3.0 ± 0.3) and (3.8 ± 0.2). The correct arm entry counts decreased with each trial in NaCN (-0.08 ± 0.1), but increased in NaOCN (0.14 ± 0.2) and saline (0.02 ± 0.03) treated rats. The change in the entry counts with each trial was significantly higher among NaCN compared to saline ($p < 0.01$) and NaOCN ($p < 0.05$) treated rats. However, no statistical difference ($p = 0.15$) was realized on entry counts with each trial between NaOCN relative to saline treated rats.

There was significant influence ($F_{2, 19} = 9.35$ $p < 0.05$) of treatment on re-entry counts (WME) into the baited arms (Fig. IIIb). The mean re-entry counts into already visited baited arms across the 10 trials in rats treated with NaOCN, NaCN and saline were (25.50 ± 7.5), (30.8 ± 5.7) and (3.8 ± 2.1), respectively. The re-entries changed in each trial, with an overall decrease among animals treated with NaOCN (-8.20 ± 3.4), NaCN (-3.2 ± 2.4) and saline (-4.7 ± 1.3). The decrease in re-entry counts was significantly higher with each trial among NaOCN treated rats compared to NaCN ($p < 0.001$) and saline ($p < 0.05$) groups. However, compared to the controls, rats treated with NaCN had marginal ($p = 0.07$) decrease in re-entries with each trial into already visited baited arms.

There was no significant influence ($F_{2, 19} = 1.70$ $p = 0.20$) of treatment on entries into un-baited arms (RME) among rats navigating RAM (Fig. IIIc). The mean entry counts into the un-baited arms across the 10 trials among rats treated with NaOCN, NaCN relative to saline were (4.3 ± 0.8), (5.1 ± 0.6) and (2.2 ± 0.7), respectively. The counts changed with each trial, with an overall decrease in rats treated with NaOCN (-0.35 ± 0.3), NaCN (-0.1 ± 0.1) and saline (-0.29 ± 0.2). The entries decreased more with each trial among NaOCN treated rats compared to NaCN and control groups, though the difference was not statistically significant.

There was no significant effect ($F_{2, 19} = 2.49$ $p = 0.12$) of treatment on RAM navigation time among experimental rats (Fig. IIId). The mean RAM navigation time across the 10 trials in rats treated with NaOCN, NaCN relative to controls during long-term memory testing were (3.73 ± 0.9), (3.64 ± 0.4) and (1.90 ± 0.1) minutes, respectively. The navigation time decreased with each trial among rats treated with NaOCN (-0.25 ± 0.03), NaCN (-0.05 ± 0.02) relative to saline (-0.0004 ± 0.1), respectively. The change in RAM navigation time was significantly ($p < 0.05$) higher with each trial in the rats treated with NaOCN compared to controls. However, a comparison between NaOCN and NaCN treated rats did not demonstrate any statistical difference ($p = 0.10$).

Discussion

We carried out comparative analyses of the toxicity behavior of cyanate relative to cyanide in laboratory animals. Animals treated with NaOCN gained weight at a slower rate relative to NaCN and controls groups. This is consistent with previous study in which rats treated with cyanate had nutritional derangement associated with loss of subcutaneous fat and organ weight loss (Blanche et al. 1974). Loss of weight, however, was not observed with our experimental paradigm despite rats fed on 85% food restricted regimen. Rodents showed no signs of weight loss such as diminishing fat on bone prominences. This was probably due the low dose of NaOCN used in our protocol. However, the finding on slow weight gain is consistent with observation seen among children population subsisting mainly on cassava

because of its poor protein and cyanogenic content (Banea-Mayambu et al. 2000; Nunn et al. 2011).

With regard to cyanide, it appears that organisms have evolved effective detoxification mechanisms minimizing its cumulative effects even with higher doses (Hayes 1967) thereby, dissipating its effect on body weight and other physiological parameters. We report no effect of cyanide on rodent weight changes a fact corroborated by its non cumulative pattern consistent with other studies among pigs and rats which showed toxicological interrelationship between cyanide, dietary protein and iodine but found no effect on body weight, food consumption, and organ weights following cyanide administration (Tewe and Maner 1982).

We further showed that radial arm maze is a valid tool that may be used to assess neuro-cognitive functions (Olton and Samuelson 1976) in animals treated with cyanate or cyanide. Our model and findings may have translational implications on cassava-associated neurotoxicity patterns and/or mechanisms. Rodents treated with NaOCN or NaCN demonstrated differential pattern in spatial learning and memory on short as well as long-term spectrum. In the initial phase of short-term memory assessment, treatment with NaOCN impaired the working memory but this was spared by NaCN treatment. This may have been due to toxicity behavior of cyanate, which may be almost instantaneously capable of adducting proteins. Cyanate has been implicated in the impairment of cognitive function in rodents (Crist et al. 1973). The mechanism through which it perturbed cognition is not well understood. However, it is known to induce protein carbamylation (Fando and Grisolia 1974), which may change the protein structure and function (Fando and Grisolia 1974; Kassa et al. 2011). Through carbamylation of brain proteins and other yet to be explored mechanisms, cyanate may affect the process of signal transduction, synaptic plasticity and memory formation (Crist et al. 1973; Fando and Grisolia 1974). Protein modification may also affect long-term potentiation, the cellular basis for learning and memory in the hippocampus (Frey et al. 1991; Fando and Grisolia 1974). There exists a parallel relationship between the extent of brain protein carbamylation with learning and retention of memory (Crist et al. 1973). NaOCN impaired motivation probably due to inhibition on appetite affecting the urge to feed as a result of toxicity as evidence by longer RAM navigation time. This may also explain the findings on body weight changes relative to cyanide and control groups.

NaCN did not affect working memory during the short-term memory training phase. Cyanide is a potent and selective neurotoxin (Mills et al. 1996) relative to NaOCN. Cyanide is also effectively detoxified and its cumulative effects are rarely seen even with higher doses (Hayes 1967). Neurological damage after cyanide exposure is attributed primarily to its hypoxic action, which results to reduction in cellular metabolism and energy through inhibition of cytochrome c oxidase (Way 1984). Lack of changes in short-term memory may be explained by efficient detoxification mechanisms and absence of cumulative patterns due to its well-evolved detoxification process. This may also, however, be a dose-dependent phenomenon. We favor the first explanation since our doses were close to the LD50 of NaCN i.e. given at sublethal levels. Our findings still retain some level of public health references as peak in cyanide exposure, together with acute signs of cyanide poisoning and lethality do precede outbreaks of konzo (Banea-Mayambu et al. 2000).

During the long-term memory testing, animals treated with NaOCN appeared to recover from poor working memory performance. This finding is difficult to explain though possibly associated with clearance of carbamoylated proteins (Crist et al. 1973). In the long run, NaCN impaired working memory, but had no effect on reference memory. This is an indication of delayed effect of cyanide on memory processes. In addition, the observed

pattern of cyanide may be explained by cumulative effects of OCN build-up levels as a result of NaCN metabolism (Swenne et al. 1996; Tor-Agbidye et al. 1999). Furthermore, it has been demonstrated that, in the long-run cyanide leads to chronic hypoxia in rats with resultant lesion producing long-term behavioral disorders such as decreased locomotor activity and decreased memory, which are partly ascribed to dopaminergic and possibly noradrenergic dysfunction affecting long-term potentiation, the cellular basis for learning and memory in the hippocampus (Speiser et al. 1990; Mathangi and Namasivayan 2000ab; Frey et al. 1991). These findings are consistent with observed short-term memory impairment 5 months after cyanide poisoning in a female individual who received antidotal treatment (Chin and Calderon 2000).

The pattern observed during both short and long-term memory assessments may be pointing to the possibility that the two types of memory may not necessarily be associated. Studies on brain lesions have suggested that the two systems may depend on different brain systems (Dudchenko 2004). The behavior and pattern of the two phases may also be explained by the differences in flexibility in stimulus-response associations and their possible sensitivity to interference in which working memory appears to be more vulnerable compared to reference memory (Dudchenko 2004). In addition, working memory (errors) have been found to be more sensitive to drug effects, suggesting that manipulation of recent, transient information is more readily affected by pharmacological changes than retrieval from long-term memory (Hodges 1996). The differential patterns of the effects of NaOCN relative NaCN may be indicative of their differential toxicity mechanisms.

The differential effect observed with cyanogenic analogs treatment on spatial learning and memory in rodents is reminiscence of complexity of the effects of toxic cassava. Children with or without konzo demonstrated poor cognitive performance compared to the control (Boivin et al. 2013), a fact that has been corroborated by our findings. A previous electroencephalography study on konzo had suggested that cassava neurotoxicity may be associated with poor cognition (Tshala-Katumbay et al. 2000). These findings (Tshala-Katumbay et al. 2000; Boivin et al. 2013) together with our findings add weight to the burden of cassava related toxicity. They do call for adequate processing of cassava prior to its human consumption. Further studies are needed to unravel the critical time of the onset of the possible deleterious effects on cognition. Both cyanide and cyanate may cause cognitive impairment in humans. Previous studies have suggested that mental retardation could be due to the effect of cyanogens on the endocrine system or because of the effect on cyanate modification of brain proteins (Cliff and Nicala 1997; Tshala-Katumbay et al. 2001; Crist et al. 1973). Further studies are needed to elucidate the biological markers that best correlate with levels of cognition deficits among cassava-reliant children. Such studies will inform choice for interventions to reduce the burden of cassava associated neurological disease.

In conclusion, NaOCN intoxication has an impact of short-term working memory functions. In contrast, NaCN tends to induce deficits in long-term working memory. It is therefore possible that cognition deficits associated with cassava cyanogenesis may reflect a dual toxicity effect of cyanide and cyanate.

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References

- Aggleton JP, Hunt PR, Rawlins JNP. The effect of hippocampal lesions upon spatial and nonspatial tests of working memory. *Behav Brain Res.* 1986; 19:133–146. [PubMed: 3964405]
- Amizet L, Pruvot G, Remy S, Kfoury M. Occupational cyanide poisoning. *BMJ Case Rep.* 2011 doi: 10.1136/bcr.09.2011.4865.
- Banea-Mayambu JP, Tylleskar T, Gitebo N, Matadi N, Gebre-Medhin M, Rosling H. Geographical and seasonal association between linamarin and cyanide exposure from cassava and the upper motor neurone disease konzo in former Zaire. *Trop Med Int Health.* 1997; 2:1143–51. [PubMed: 9438470]
- Banea-Mayambu JP, Tylleskar T, Tylleskar K, Gebre-Medhin M, Rosling H. Dietary cyanide from insufficiently processed cassava and growth retardation in children in the Democratic Republic of Congo (formerly Zaire). *Ann Trop Paediatr: Int Child Health.* 2000; 20:34–40.
- Blanche PA, Yuet WK, David GN. Toxic Effects of High-Dose Cyanate Administration in Rodents. *Blood.* 1974; 43:69–77. [PubMed: 4809099]
- Boivin MJ, Okitundu D, Makila-Mabe Bumoko G, Sombo MT, Mumba D, Tylleskar T, Page CF, Tamfum Muyembe JJ, Tshala-Katumbay D. Neuropsychological effects of konzo: A neuromotor disease associated with poorly processed cassava. *Paediatr.* 2013; 131:e1231–1239.
- Carlsson L, Mlingi N, Ronquist G, Rosling H. A specific and sensitive method for determination of linamarin in urine. *Nat toxins.* 1995; 3:378–382. [PubMed: 8581323]
- Chin RG, Calderon Y. Acute cyanide poisoning: A case report. *J Emerg Med.* 2000; 18:441–445. [PubMed: 10802422]
- Cliff J, Muquingue H, Nhassico D, Nzwalo H, Bradbury JH. Konzo and continuing cyanide intoxication from cassava in Mozambique. *Food Chem Toxicol.* 2011; 49:631–635. [PubMed: 20654676]
- Cliff J, Nacala D. Long term follow-up of konzo patients. *Trans Royal Soc Trop Med.* 1997; 91:447–449.
- Dudchenko PA. An overview of the tasks used to test working memory in rodents. *Neurosci and Behav Rev.* 2004; 28:699–709.
- Eichenbaum H, Otto T, Cohen NJ. Two functional components of the hippocampal memory system. *Behav Brain Sci.* 1994; 17:449–518.
- Essers AJA, Alsen P, Rosling H. Insufficient processing of cassava induced intoxications and the paralytic disease konzo in a rural area of Mozambique. *Ecol Food and Nutr.* 1992; 27:17–27.
- Frey U, Mathies H, Reymann KG. The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long term potentiation in the rat CA1 region in vitro. *Neurosci Lett.* 1991; 129:111–116. [PubMed: 1833673]
- Hayes WT. The 90-dose LD50 and a chronicity factor as measures of toxicity. *Toxicol and Appl Pharmacol.* 1967; 11:327–335.
- Hodges H. Maze procedures: the radial-arm and water maze compared. *Cogn Brain Res.* 1996; 3:167–181.
- Kassa RM, Kasensa NL, Monterroso VH, Kayton RJ, Klimek JE, David LL, Lunganza KR, Kayembe KT, Bentivoglio M, Juliano SL, Tshala-Katumbay DD. On the biomarkers and mechanisms of konzo, a distinct upper motor neuron disease associated with food (cassava) cyanogenic exposure. *Food Chem Toxicol.* 2011; 49:571–578. [PubMed: 20538033]
- Kelsey JE, Vargas H. Medial septal lesions disrupt spatial, but not nonspatial working memory in rats. *Behav Neurosci.* 1993; 107:565–574. [PubMed: 8397861]
- Kuramoto T, Nakanishi S, Ochiai M, Nakagama H, Voigt B, Serikawa T. Origins of albino and hooded rats: implications from molecular genetic analysis across modern laboratory rat strains. *PLoS ONE.* 2012; 7:e43059. [PubMed: 22916206]
- Mathangi DC, Namasivayam A. Effect of cassava consumption on open-field behavior and brain neurotransmitters in albino rats. *Physiol & Behav.* 2000; 70:89–93. [PubMed: 10978482]
- Mathangi DC, Namasivayam A. Effect of Chronic Cyanide Intoxication on Memory in Albino Rats. *Food and Chem Toxicol.* 2000; 38:51–55. [PubMed: 10685013]
- Mills EM, Gunasekar PG, Pavlakovic G, Isom GE. Cyanide-Induced Apoptosis and Oxidative Stress in Differentiated PC 12 Cells. *J Neurochem.* 1996; 67:1039–1046. [PubMed: 8752110]

- Nunn PB, Lyddiard JR, Christopher Perera KP. Brain glutathione as a target for aetiological factors in neuroleptism and konzo. *Food Chem Toxicol.* 2011; 49:662–667. [PubMed: 20816718]
- Nzwalo H, Cliff J. Konzo: From poverty, cassava, and cyanogen intake to toxico-nutritional neurological disease. *PLoS Negl Trop Dis.* 2011; 5:e1051. [PubMed: 21738800]
- Okafar PN, Okorowkwo CO, Maduagwu EN. Occupational and dietary exposures of humans to cyanide poisoning from large-scale cassava processing and ingestion of cassava foods. *Food and Chem Toxicol.* 2002; 40:1001–1005. [PubMed: 12065222]
- Olton DS, Samuelson RJ. Remembrance of places passed: Spatial memory in rats. *J Exp Psychol: Anim Behav Proc.* 1976; 2:97–116.
- Olton DS. The radial arm maze as a tool in behavioral pharmacology. *Physiol & Behav.* 1985; 40:793–797. [PubMed: 3313453]
- Rosling H. Measuring effects in humans of dietary cyanide exposure from cassava. *Acta Hort.* 1994; 375:271–283.
- Rosling H, Gessain A, de Thé G, Ebondo N, Banea M, Bikangi N, Kinjanja K, Nunga M. Tropical and epidemic spastic paraparesis are different. *Lancet.* 1988; 8596:1222–1223. [PubMed: 2897030]
- Rosling, H.; Tylleskar, T. *Tropical Neurology.* Saunders; London: 1995. Konzo an epidemic upper motor neuron disease associated to cassava and agro-ecological collapse in Africa; p. 353–364.
- Speiser Z, Amitz-Zonder J, Ashkenazi R, Gitter S, Cohen S. Central catecholaminergic dysfunction and behavioural disorders following hypoxia in adult rats. *Behav and Brain Res.* 1990; 37:19–27. [PubMed: 2310491]
- Spencer PS. Food toxins, AMPA receptors, and motor neuron diseases. *Drug Metab Rev.* 1999; 31:561–587. [PubMed: 10461541]
- Swenne I, Eriksson UJ, Christoffersson R, Kagedal B, Lundquist P, Nilsson L, Tylleskar T, Rosling H. Cyanide detoxification in rats exposed to acetonitrile and fed a low protein diet. *Fundam Appl Toxicol.* 1996; 32:66–71. [PubMed: 8812227]
- Terragon E, Lopez L, Ros-Bernal F, Yuste JE, Ortiz-Cullera V, Martin E, Schenker E, Aujard F, Bordet R, Richardson JC, Herrero MT. The Radial Arm Maze (RAM) for the evaluation of working and reference memory deficits in the diurnal rodent *Octodon degus*. *Proc Meas Behav.* 2012
- Tewe OO, Maner JH. Cyanide protein and iodine interaction in the physiology and metabolism of rats. *Food Chem.* 1982; 9:195–204.
- Tor-Agbidye J, Palmer VS, Lasarev MR, Craig AM, Blythe LL, Sabri MI, Spencer PS. Bioactivation of cyanide to cyanate in sulfur amino acid deficiency: relevance to neurological disease in humans subsisting on cassava. *Toxicol Sci.* 1999; 50:228–235. [PubMed: 10478859]
- Tshala-Katumbay D, Eeg-Olofsson K, Tylleskar T, Kazadi-Kayembe T. Impairments, disabilities and handicap pattern in konzo - a non-progressive spastic para/tetraparesis of acute onset. *Disabil and Rehabil.* 2001; 23:731–736.
- Tshala-Katumbay D, Lukusa VM, Eeg-Olofsson K. EEG findings in Konzo: a spastic para/tetraparesis of acute onset. *Clin Electroencephalogr.* 2000; 31:196–200. [PubMed: 11056842]
- Tylleskär T, Banea M, Bikangi N, Fresco L, Persson LÅ, Rosling H. Epidemiological evidence from Zaire for a dietary aetiology of konzo, an upper motor neuron disease. *Bull World Health Organ.* 1991; 69:581–590. [PubMed: 1959159]
- Wenk G, Sweeney J, Hughey D, Carson J, Olton D. Choline acetyltransferase inhibition does not impair radial maze performance in rats. *Pharmacol Biochem Behav.* 1986; 25:521–526. [PubMed: 3774818]

Fig. 1. Weight (g) change in rodents treated with normal saline, NaCN or NaOCN. Animals treated with NaOCN gained weight slowly ($p < 0.05$) relative to controls but compared to NaCN treated rats, the change was not statistically different ($p=0.59$).

Fig. IIa.

Correct arm entry counts into 8 baited arms across the 8 trials among rats treated with NS, NaCN or NaOCN. The mean correct arm entry counts across the trials in NaOCN (3.6 ± 0.7) were fewer compared to NaCN (4.1 ± 0.4) and saline (6.2 ± 0.6) treated rats. The counts decreased significantly ($p < 0.05$) with each trial in NaOCN (-0.19 ± 0.2) compared to an increase in both NaCN (0.13 ± 0.2) and control (0.16 ± 0.3) groups. However, no statistical difference ($p=0.8$) between NaCN and controls was demonstrated.

Fig. IIb.

Re-entry counts into already visited arms (WME) among rats treated with NS, NaCN or NaOCN. The mean re-entry counts across the 8 trials in NaOCN (85.05 ± 7.7) were higher compared to NaCN (77.98 ± 4.4) and control (26.11 ± 2.6) treated rats. The counts increased with each trial among NaOCN (8.40 ± 2.2) compared to NaCN (-1.18 ± 0.4) ($p < 0.05$) and saline (-3.21 ± 0.9) ($p < 0.01$) treated rats. However, no statistical difference ($p = 0.5$) was realized with each trial between NaCN and saline treated rats.

Fig. IIc.

RAM navigation time into 8 baited arms among rats treated with NS, NaCN or NaOCN. The mean RAM navigation time across the trials among rats treated with NaOCN (6.26 ± 0.3) was longer compared to NaCN (4.72 ± 0.1) and controls (2.82 ± 0.3) minutes. The time increased with each trial in NaOCN (0.25 ± 0.03) relative to NaCN (0.02 ± 0.01) and controls (-0.01 ± 0.02) ($p < 0.05$). However, there was no statistical difference ($p = 0.23$) between NaOCN and NaCN treated animals with each trial.

Fig. IIIa.

Correct entry counts into 4 baited arms of RAM in rats treated with NS, NaCN or NaOCN during long-term memory assessment. The mean arm entry counts across the trials among rats treated with NaOCN (3.1 ± 0.6) and NaCN (3.0 ± 0.3) were fewer relative to controls (3.8 ± 0.2). The entry counts however, decreased with each trial in NaCN (-0.08 ± 0.09) compared to NaOCN (0.14 ± 0.16) ($p < 0.01$) and control (0.02 ± 0.03) ($p < 0.05$) treated rats. However, no statistical difference ($p = 0.15$) was realized with each trial between NaOCN and controls.

Fig. IIIb.

Re-entry counts into visited baited arms (WME) by rats treated with NS, NaCN or NaOCN during long-term memory assessment. The mean re-entry counts across the trials among rats treated with NaCN (30.8 ± 5.7) were higher compared to NaOCN (25.50 ± 7.5) and controls (3.8 ± 2.1). The re-entry counts decreased with each trial in NaOCN (-8.20 ± 3.4), compared to NaCN (-3.2 ± 2.4) ($p < 0.001$) and vehicle (-4.7 ± 1.3) ($p < 0.05$), treated rats. A marginal statistical difference ($p = 0.07$) was realized with each trial between NaCN relative to controls.

Fig. IIIc.

Entry counts into un-baited arms (RME) by rats treated with NS, NaCN or NaOCN during long-term memory assessment. The mean entry counts into the un-baited arms across the 10 trials among rats treated with NaCN, (5.1 ± 0.6) was higher compared to NaOCN (4.3 ± 0.8), and controls (2.2 ± 0.7). The counts decreased more with each trial in NaOCN (-0.35 ± 0.3), compared to NaCN (-0.1 ± 0.1) and controls (-0.29 ± 0.2), respectively, but no statistical difference was yielded.

Fig. IIIId.

RAM navigation time among rats treated with NS, NaCN or NaOCN during long-term memory assessment. The mean RAM navigation time across the trials in rats treated with NaOCN (3.73 ± 0.9) and NaCN (3.64 ± 0.4) were longer compared to controls (1.90 ± 0.1) minutes. The time decreased significantly ($p < 0.05$) with each trial among NaOCN (-0.25 ± 0.03) relative to saline (-0.0004 ± 0.1) treated rats. However, there was no statistical difference ($p = 0.10$) realized with each trial between NaOCN and NaCN (-0.05 ± 0.02) treated rats.