

Motor and memory function in rat models of cyanide toxicity and vascular occlusion induced ischemic injury

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Abstract

Although oxidative stress is characteristic of global vascular occlusion and cyanide toxicity, the pattern of cerebral metabolism reconditioning and rate of progression or reversal of neural tissue damage differ for both forms of ischemia. Thus, it is important to compare cognitive and motor functions in both models of ischemia involving cyanide treatment (CN) and vascular occlusion (VO).

Adult Wistar rats ($N=30$) were divided into three groups; VO ($n=12$), CN ($n=12$) and Control-CO ($n=6$). The CN was treated with 30 mg/Kg of potassium cyanide (KCN); VO was subjected to global vascular occlusion-both for duration of 10 days. The control (CO) was fed on normal rat chow and water for the same duration. At day 10, the test and control groups (CN, VO and CO) were subjected to motor function tests (Table edge tests and Open Field Test) and memory function tests (Y-Maze and Novel object recognition) while the withdrawal groups CN-I and VO-I were subjected to the same set of tests at day 20 (the withdrawal phase).

The results show that both cyanide toxicity and vascular occlusion caused a decline in motor and memory function when compared with the control. Also, the cyanide treatment produced a more rapid decline in these behavioral parameters when compared with the vascular occlusion during the treatment phase. After the withdrawal phase, cyanide treatment (CN-I) showed either an improvement or restoration of motor and memory function when compared to the CN and control. Withdrawal of vascular occlusion caused no improvement, and in some cases a decline in motor and memory function.

In conclusion, cyanide toxicity caused a decline in motor and memory function after the treatment while vascular occlusion caused no significant decline in cognition and motor function at this time. After the withdrawal phase, the effect of cyanide toxicity was reduced and significant improvements were observed in the behavioral tests (motor and cognitive), while a decline in these functions were seen in the vascular occlusion group after this phase.

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1. Introduction

Cerebral blood flow is an important part of brain metabolism as it is evident from stroke and metabolic

poisoning, such as cyanide toxicity [1]. The blood vessels are specialized structures and can adjust blood flow patterns to meet regional requirements of various parts of the brain during normal and oxygen deprived states [1,2]. All of these are reflected in cognition, memory, movement and neurological activities expressed in the behavior of organisms both in normal and diseased states.

Stroke is a major cause of death and disability in humans [3,4]. It is often life threatening with most patients suffering from disabilities such as palsy, memory loss and

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speech impairment requiring extra care and management therapies [5,6]. Stroke can also be described as an acute event with long term economic, psychological and social impacts on the patients as they often suffer from isolation, mood disturbances, communication difficulties and movement disorders experienced by these patients [7]. Cyanide poisoning on the other hand induces ischemia by inhibition of mitochondria respiration. The effect is rapid and death could occur within hours in extreme cases [8]. In acute slow treatment or exposure, neurological symptoms, neuropathies and degenerative effects have been described [8,9]. Osuntokun and co-workers in 1981, described several degenerative changes in the population exposed to cassava based diet; leading to loss of cognition, Parkinson like symptoms, retrobulbar neuritis, tropical ataxic neuropathy (TAN) and spastic endemic paraparesis (Konzo) [10]. The incidence of these diseases is often associated with exposure to cyanide from the environment, industrial waste and consumption of cyanophoric plants [11,12].

The role of diet in stroke and cyanide poisoning has been described by several authors [13–16]. Fruits and vegetables, being the source of flavonoids, phenols, carotenoids, and anthocyanin are important anti-inflammatory and antioxidants in stroke preventions and management [17,18]. Consumption of protein rich diets have also been reported to reduce the severity of cyanide poisoning and neurodegenerative changes in cassava endemic regions by aiding the removal of cyanide as thiocyanate through the interaction between CN and sulphur containing amino acids [8,14–16]. In both forms of ischemia, that is, vascular occlusion (model for stroke) and cyanide toxicity, induction of oxidative stress, degenerative changes, cognitive impairment, movement impairment and neuropathies are common [19,20]. This is suggestive that ischemia is associated with both anatomical and behavioral deficits [21,22]. MRI studies have shown changes with basic neural networks that control and affect behavior, movement, speech, and cognition post ischemia [19,23,24]. Behavioral analysis in rodent models of GVO and cyanide treatment is important for explaining the underlying differences in the effect of the GVO and cyanide toxicity on memory and motor functions. In this study we have studied, comparatively, the variations in motor and cognitive functions in cyanide toxicity and vascular occlusion following a short-term treatment and withdrawal of the treatments.

2. Materials and methods

2.1. Animal preparation and treatment

Male adult Wistar rats ($N=30$) were procured from the Animal Holding Facility of Afe Babalola University. The animals were then divided into three groups; $n=12$, $n=12$ and $n=6$ (average weight was 250 g). The animals were allowed to acclimatize for 12 days under standard laboratory

conditions and controlled environment of 12 h light/dark cycle with free access to food and water.

2.2. Treatment

A group of male rats ($n=12$) were treated with orally administered potassium cyanide (KCN) at 30 mg/Kg body weight (BW) of KCN for 10 days by gavage (CN). A second set of adult male rats ($n=6$) were fed on normal rat chow and treated with normal saline for the same duration (Control; CO). A separate group of $n=12$ animals were subjected to transient occlusion of both carotid arteries, basilar artery and brachiocephalic vein with use of elastic neck cuff placed around the neck. The neck cuff was adjusted until the pressure of blood measured from the surface was about 30 mmHg above the neck cuff [36,37]. The use of elastic material as the neck cuff facilitated local irritation and inflammation around the occluded region by the 5th day. This was done for a total duration of 10 days following which the behavioral tests were administered to $n=6$ animals each in the VO, CN and Control groups. The animals were handled carefully during the treatment using approved protocols by the institutional Animal Care and Ethics Committee. On the 11th day, KCN treatment was discontinued and the neck cuffs removed in $n=6$ animals each, remaining in the VO and CN groups. These groups were renamed VO-I and CN-I as they represent the withdrawal groups for vascular occlusion and cyanide treatment respectively. At the end of the withdrawal phase, that is day 20, animals were subjected to motor and memory function tests.

2.2.1. Novel object recognition test (NOR)

Object recognition memory function was assessed using NOR test. A 75 cm × 50 cm × 30 cm transparent box was used. Three days prior to the habituation sessions, the rats were exposed to the box to familiarize with the environment. On the test days, they were exposed to two identical objects to acclimatize with for 5 min which is termed trial 1 (T1). The rats were then put inside a cage with food and water. Thirty minutes later, i.e. inter trial interval (ITI), the rats were placed back inside the box with one of the object replaced by a novel one for 5 min. The time used in rearing on the old (old time) and new object (new time) was recorded using a video camera suspended about 60 cm away from the habituation box. Animal rearing was measured when the nose of the animal is less than 2 cm from the object while sitting on the object is not considered [25].

$$\text{Memory index(\%)} = \frac{\text{Time spent on new object}}{\text{Total time spent on rearing both old and new object}} \times 100$$

2.2.2. Y-Maze

This is done to check the spatial working memory of the rats. The rats were placed facing the edge and were to make their arm decision. The duration for the test was 10 min. The

percentage alternation was recorded. Visiting the three arm consecutively was termed right decision (right) and visiting one arm twice in three alternation was termed wrong decision (wrong). Percentage alternation (Memory index) was calculated for the rats.

$$\% \text{ alternation} = \frac{\text{No. of right decision}}{\text{No. of total arm entry} - 2} \times 100$$

2.2.3. Open field test

The effect of ischemia on motor activities of the rats was assessed according to a modified method of Swiergiel and Dunn, 2007 [26]. The rats were placed individually in the open field box and the number of lines crossed was counted manually for a period of 5 min.

2.2.4. Table edge test

The animals were placed equidistant from the edge of a table. The holding platform was about 10 cm above the table edge. As the animals extend forward to mount the table edge, the number of limbs extended was noted. Also, the duration spent on the platform before mounting the table edge was also noted.

2.2.5. Statistical analysis and cell count

Data obtained was plotted using *GraphPad Prism* (Version 5.0). All behavioral data are presented as mean \pm SEM.

3. Results

3.1. NOR

The NOR test was used to assess working and spatial memory in the two models of ischemia tested in this study. The memory index declined considerably in the CN treatment when compared with the control ($P < 0.01$). Following withdrawal, an improvement in the memory index was observed in the CN-I group when compared to the CN ($P < 0.01$) and control ($P < 0.05$) (Fig. 1). In the vascular occlusion group, the memory index was not significantly reduced when compared with the control after the treatment phase. However, no significant change was observed in the memory index of the VO treatment following withdrawal as seen in the VO-I group (NS).

3.2. Y-Maze

This was used to assess cognitive function and exploratory memory in the animals. In the VO, no major alteration was observed in the memory index when compared with the control (Fig. 2). After the withdrawal period, a decline was observed in the memory index of vascular occlusion treatment (VO-I) when compared to VO ($P < 0.01$). However, in the CN treatment, a decline in the memory index was observed when compared with the control and the VO

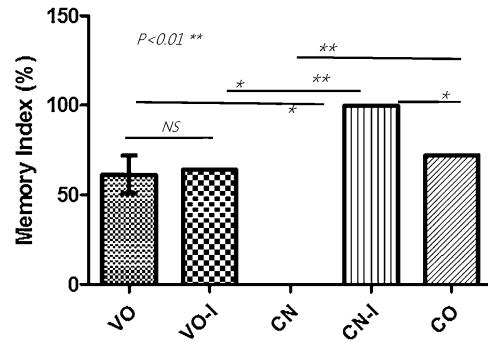


Fig. 1. Bar chart representing the memory index in NOR. The memory index was least in the CN treatment and improved following withdrawal (CN-I; $P < 0.01$). The memory index was reduced in the VO treatment and no major improvement was observed in the VO-I (NS – no significance). All values are expressed as mean \pm SEM. Error was zero for the VO-I, CN, CN-I and CO.

($P < 0.05$). After the withdrawal phase no significant change in memory index was observed when compared with the control and other treatment groups.

3.3. OFT

The frequency of lines crossed was calculated in the open field area (OFA) to measure ambulatory movement. The CN showed the least exploratory activity in the OFA when compared with the control ($P < 0.05$) a phenomenon that improved after the withdrawal phase (Fig. 3; CN-I) when compared with the control ($P > 0.05$). In the VO treatment, the number of lines crossed increased when compared to the control and was relatively unchanged following withdrawal (VO-I).

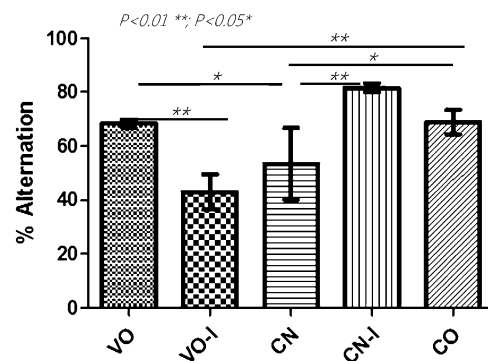


Fig. 2. The memory index in Y-Maze. This was calculated as percentage alternation between the arms of the maze. There was a reduction in the memory index of the CN treatment but not the VO treatment when compared with the control ($P < 0.05$). However, following withdrawal, the VO treatment showed a decline as seen in the VO-I ($P < 0.01$). An improvement in memory index was observed in the CN after the withdrawal period as demonstrated in the CN-I ($P < 0.001$).

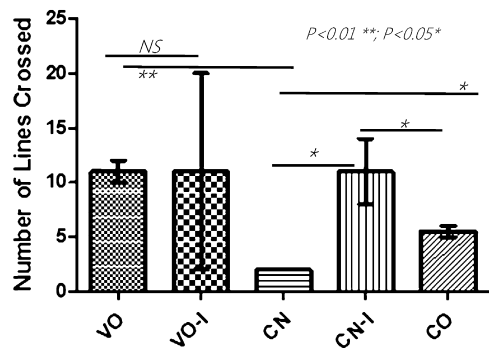


Fig. 3. Bar chart representing the number of lines crossed in OFT. A reduction in the frequency of line crossing was observed in the CN treatment when compared with the VO ($P < 0.01$) and the control ($P < 0.05$). Following withdrawal the mean frequency of lines crossed remained relatively the same for the VO and VO-I (NS). In both the VO and VO-I the number of lines crossed were higher than that of the control ($P < 0.05$) and the cyanide treatment ($P < 0.01$). However, following 10 days of withdrawal, an increase in ambulatory movement was observed in the CN-I group when compared with the CN and the control ($P < 0.05$).

3.4. Table edge test

The time taken before the animal extends its limb to mount the table edge and the number of limbs extended to the edge of the table were recorded. An average of 1 limb was extended in the VO group and was unchanged in the VO-I group after the withdrawal phase (NS). In both the VO and VO-I, the number of limbs extended were less than that of the control ($P < 0.05$). The CN treatment showed no decrease in the number of limbs of extended when compared with the control (NS). An improvement in movement coordination was observed in the CN-I after the withdrawal phase; the CN-I extended more limbs than the CN ($P < 0.01$). Considering the time taken before the limbs were extended, the control group recorded the least time and therefore showed the highest coordination and motor processing. The VO and CN treatment showed an increase in the latency when compared with the control ($P < 0.05$). Following withdrawal, the VO-I and CN-I showed further increase in the time taken before limbs were extended when compared versus VO and CN respectively ($P < 0.05$). The latency after the withdrawal phase for VO-I ($P < 0.05$) and CN-I ($P < 0.01$) was also significant when compared with the control.

4. Discussion

Taken together, the outcomes of our findings confirm that cyanide toxicity and vascular occlusion caused significant changes in motor and cognitive functions after the treatment and withdrawal phases. In furtherance, the changes induced by cyanide treatment differ from those induced by vascular occlusion during these phases for the duration adopted in this study. A reduction in the memory index observed in the CN treatment, lower than that recorded in the VO treatment at the

end of the treatment phase, thus, the effects of cyanide treatment can be described as being more significant on memory consolidation after the treatment phase when compared with the VO ($P < 0.05$) and control ($P < 0.01$).

After the treatment phase, vascular occlusion showed no significant change in memory consolidation in NOR as the withdrawal group for this treatment (VO-I) showed no prominent change in memory function at day 20 (NS) (Fig. 1). However, the CN-I showed a drastic improvement in memory consolidation when compared to the CN ($P < 0.01$) and control ($P < 0.05$). These outcomes suggests that, although the effect of CN was more severe on memory consolidation after the treatment phase, a decline when compared to VO, reversal and improvement in memory function after the withdrawal phase was also more significant in the CN-I treatment when compared with the VO-I ($P < 0.05$) and control ($P < 0.05$).

To further clarify these findings, the animals were subjected to a separate task to measure cognition using the Y-Maze (Fig. 2). In this task the animals were expected to explore new arms of the maze more than the familiar arms. This was expressed as the percentage of alteration between the old and the new arms of the maze to represent the memory index. After the treatment phase, the VO showed no decline in memory consolidation when compared with the control, while the CN treatment showed a prominent decline in memory processing when compared with the control ($P < 0.05$) and VO ($P < 0.05$). Similar to our findings in the NOR, the VO withdrawal (VO-I) showed a decline in the memory index when compared with the control ($P < 0.01$) and the VO ($P < 0.01$) after the withdrawal phase. In cyanide treatment (CN), a decline in memory index was recorded for the treatment when compared with the control. Similar to our observations in the NOR for the spatial working memory, the CN-I also showed an improved cognition in the Y-Maze task when compared with the CN ($P < 0.001$). Consequently, a trend can be described for the differential memory function between cyanide and vascular occlusion; VO induced a reduction in memory function, that was either constant (NOR) or reduced (Y-Maze) following withdrawal while CN induced a decline in memory function after the treatment phase and improved significantly after the withdrawal phase. The decline observed in VO after the treatment phase were also not as prominent as those observed in the CN treatment. Our findings suggest that treatment for a short period could aid memory function in cyanide treatment (Figs. 1 and 2) after the withdrawal phase rather than cause impairment of short term memory as widely reported [14,15,21–24]. Comparing the VO-I and VO showed that withdrawal neither reversed nor improved memory deficit in this treatment, thus reflecting the cortical nature of the damage induced by the vascular occlusion [38,41]. Increased rate of old object exploration in the treatment phase of this study is supported by other studies on the hippocampus which suggests enhanced object exploration post hippocampal lesion although increased exploration rate does not necessarily imply improved cognition [27–29] as observed in the VO and VO-I. This proposition is further

supported by the improved memory function observed after the withdrawal phase of the CN-I, although exploration time was less, the animals explored the new object more than the old object, thus, an improved memory consolidation reflecting a reversal in cortical damage after the withdrawal phase.

Motor impairment is often associated with cyanide toxicity and vascular occlusion. Acute intoxication by potassium cyanide did induce basal ganglia lesions on MRI. The finding of Osuntokun, 1976, suggests cortical degeneration is associated with both memory and movement; thus suggesting that motor function loss in cyanide treatment may be due neuronal loss at cortical and subcortical areas [12,14–16,30]. After the treatment phase, the OFT outcome indicates that the VO group suffered motor impairment compared with the control (Fig. 3). After the withdrawal phase, no improvement or further decline was observed in the ambulatory motor function in the open field area (OFA). Similar to the findings on memory function, the CN treatment showed a decline in the number of lines crossed in the OFA when compared to the control and VO. After the withdrawal phase, an improvement was observed in the motor function of the CN-I – higher than those observed in the VO-I, VO, CN and control. This was further checked by using a non-exploratory motor function to determine the time taken before extension of limbs and the number of limbs extended to mount a table edge (motor coordination).

Similar to our findings in the OFT, the VO treatment showed a reduction in movement coordination by extending an average of 1 limb to mount the table edge; also, the number of extended limb did not change after the withdrawal phase. Comparing this with the cyanide treatment, the CN extended on the average, 2 limbs in the treatment phase and extended 3 limbs to mount the table edge after the withdrawal phase (CN-I; Fig. 4). The control spent the least duration before the first limb mounted the table edge, thus showing a rapid motor response. An increase in time spent on platform was observed in the CN and VO treatment thus depicting a delayed motor consolidation (Fig. 5). After the withdrawal period, a further delay was observed in both VO-I and CN-I, although VO-I showed an increase in the delay time versus the CN-I. We can conclude from these findings that the effect of cyanide treatment and vascular occlusion both caused a reduction in motor function. CN recorded a rapid onset decline in motor function which improved after the withdrawal phase while the decline in VO-related motor function was either constant or declined after the withdrawal phase.

The pattern observed from the motor and memory function can further be supported by evidence from cellular studies which described the role of mild oxidative stress in neurogenesis and enhancement of synaptic activity through remodeling of dendritic spines [38,39]. The oxidative stress induced through reactive oxygen specie (ROS) production can also serve as signaling molecules which promotes post synaptic events involving recruitment of receptors. These findings further supports the idea that oxidative stress, which has been closely linked with degenerative diseases can also function as

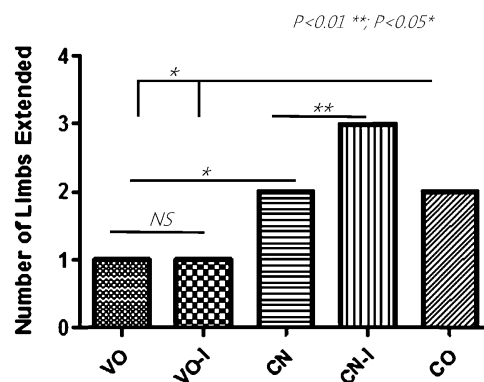


Fig. 4. Table edge test expressed as the number of limbs extended when the animal was placed close to the edge of a table. The cyanide treatment extended more limbs (2) to mount the table edge. In most cases in the VO treatment Nil or 1 limb was extended. This represents a reduction when compared with the CN ($P < 0.05$). This was further increased upon withdrawal in the CN-I (3) representing an increase in motor coordination significant at $P < 0.01$. The number of limbs extended in the VO was lesser than that of the control and remain relatively unchanged post withdrawal ($P < 0.05$). Error was zero for all groups.

regulatory molecules that can promote synaptic function by acting on inhibitory synaptic sites both in the brain and neuromuscular junction [38,40]. Other studies have also demonstrated that autophagic mechanism induced by ROS produced from the mitochondria, at physiologically favorable concentrations, can activate the JNK signaling pathway, thereby regulating synaptic function and growth [39]. We hypothesize that the decline in memory and motor consolidation observed after the treatment phase in both CN and VO reflects the effect of ROS dependent synaptic damage or aging. After the withdrawal phase, the cyanide dependent mitochondria ROS production was reduced to physiologically favorable concentration that aided synaptic strengthening. The persistent decline in the VO could have resulted from prolonged changes to the blood vessels and longer duration required for remodeling of the cerebral circulation to balance oxygen supply or reduce ROS to such a physiologically favorable concentration.

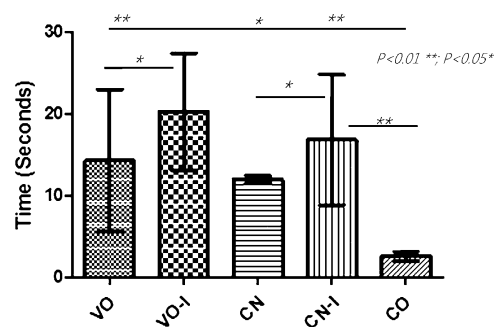


Fig. 5. Duration before the first limb was extended at the table edge. The control group showed the least duration of limb extension which corresponds to the fastest duration recorded during the test ($P < 0.01$ when compared with VO and CN). Following withdrawal, the VO-I and CN-I showed further delay before the first limb was extended toward the table edge when compared to the VO ($P < 0.05$) and the CN ($P < 0.05$) respectively.

To accurately represent the behavioral parameters, certain factors were considered. Examples of such factors include the shape, size and color of the object to be identified [31]. To account for this variations, in this study, the following modifications were made; presentation of one object during the familiarization phase, simultaneous presentation of two object during the test phase, the two objects were of same size and dimension but had two different contrasting colors (bright red and white), each animal was tested twice post the familiarization state and lastly, the same open field area was used for the familiarization and the test phases [30–32]. In order to ensure non-reinforced object recognition during the exploration, the animals were also positioned equidistant from the novel object and the object used during the familiarization phase. This design enabled us to compare directly the exploration time in the test phases for the control, GVO and CN treatments (and withdrawal groups post treatment). Since movement impairment is expected in both the VO and CN treatment, the time during the testing phase was extended. Rats were also used for the experiments and require more time to respond and move in the open field area [32].

Using the modified NOR described above, the study was able to combine the open field test (motor) with the NOR test by using the same open field area for this experiment similar to the procedure described by Zhao et al. [33]. In furtherance, enhanced cognition and motor test in the study design allowed for comparison of the effect of GVO and cyanide toxicity on memory impairment and motor dysfunction; which is often associated with ischemic injury [34]. The mechanism of GVO and CN induced memory alteration remains relatively unknown. Although cyanide has been known to cause carbonylation of cerebral proteins thereby disrupting synaptic functions and cognition, different cyanide salts creates effect at different levels. Even at high concentrations, cyanide may be detoxified and its effect might not be seen for a short-term treatment [14]. The findings of Kimani et al., 2014 suggests that memory function was not affected during the memory training phase; improved short memory may be due to an effective detoxification during the course of the treatment [13,14,22]. Studies have shown that cyanide and reduced blood supply to neural tissues severely distort neurotransmissions. From our findings, the CN and VO treatment groups did exhibit a decline in the ambulatory walk (Fig. 3) which is an indication of series of modified cortical output such as short interval cortical inhibition (SICI) in motor neurons [35] – which resulted from the hypoxic assault [28]. In the withdrawal phase, we observed that there was a reversal and improvement only in the CN-I group and not in the VO-I treatment group (Figs. 3–5). It shows that motor function recovery occurred only in the cyanide treatment withdrawal groups, an indication that detoxification of cyanide occurred rapidly after stoppage of cyanide treatment. In vascular occlusion, reduced blood flow to the brain tissues is capable of causing permanent cortical damages that were not reversed after the withdrawal of the occlusion. The damage to cortical neurons in ischemia varies for the model of ischemia and the

region of the brain affected. Previous studies have described a variation in regional cytotoxic pathways in stroke and in cyanide toxicity.

5. Conclusion

In conclusion, both cyanide toxicity and vascular occlusion caused a decline in motor (table edge test and OFT) and memory function (NOR and Y-Maze) when compared with the control. Also, the cyanide treatment produced a more rapid decline in these behavioral parameters when compared with the vascular occlusion during the treatment phase. After the withdrawal phase, cyanide treatment (CN-I) showed either an improvement or reversal in motor and memory function when compared to the CN and control. Withdrawal of vascular occlusion caused either non-improvement or a decline in motor and memory function.

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