Transient middle cerebral artery occlusion prevents habit formation in C57Bl/6J mice

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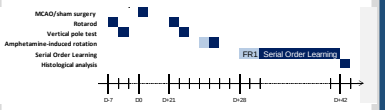
Although functional assessment is of paramount importance in preclinical stroke research, cognition has not been thoroughly investigated in animal models of cerebral ischemia. In human patients however, neuropsychological examinations frequently underline stroke-related alterations to cognition. Among them, deficits in procedural learning has been pinpointed as a common cognitive marker of subcortical stroke [1]. Surprisingly, procedural learning has never been assessed in animal stroke models, while operant conditioning offers numerous procedures to do so. **In this work, we thus used a simple Serial Order Learning (SOL) task [2] to characterize procedural learning after transient middle cerebral artery occlusion (MCAO) in mice; furthermore, we used accelerated rotarod, vertical pole test and amphetamine-induced rotation test to further assess the consequence of MCAO on sensory and motor functioning.**

Methods

Surgery. On D0, 27 eight weeks old male C57/Bl6J mice underwent 30-min MCAO or sham surgery. MCAO (n=11) was induced by inserting a 0.21 mm thick silicon-coated monofilament into the right common carotid artery, under 2% isoflurane anesthesia. Sham mice underwent the same procedure without filament insertion (n=16). **Rotarod.** In the rotarod test, mice had to stay on an accelerated rotating rod (4 to 40 rpm). Performance was averaged over three trials on D-7 (pre-surgery baseline) and on D+21. **Vertical pole test.** For this test, mice were place head upward on a vertical wooden pole. Both the time to turn (t-turn) and the time to reach the floor (t-total) were recorded over three trials on D-6 (pre-surgery baseline) and on D+22. **Amphetamine-induced rotation.** On D+25, mice were injected with d-amphetamine(5 mg/kg i.p.). Twenty minutes later, they were placed in a 50 x 50 cm white arena. 360° rotation behaviors were analyzed with a videotracking software (Viewpoint). **Serial Order Learning.** After acquisition of the operant response, food-deprived mice underwent 12 sessions of SOL in operant boxes equipped with two levers and a food-tray (Meds-Associates). At each trials, both right and left levers were available. Only the left – right lever-presses sequences were reinforced by a food reward (20 mg Noyes pellet). Other sequences were not reinforced. An 8 s inter-trial interval separated two trials. Sessions stopped once 40 rewards have been delivered or after an hour, whichever came first. **Anti-NeuN immunohistochemistry.** 50 µm thick brain sections were incubated overnight in primary mouse-anti-NeuN IgG (Chemicon), then in secondary Horse-Anti-Mouse Biotinylated IgG (Chemicon). 3,3'-diaminobenzidine (Sigma) was used as a chromogen. Infarct volume was then calculated by multiplying the average of two successive infarct areas by the distance between them.



Results

Rotarod. A Student t-test revealed a significant difference between groups on D+21 (t_{26}=2.191; p=0.038). **Vertical pole test.** While the t-turn was significantly longer in MCAO mice according to a Student t test (t_{24}=-2.151; p=0.041), the t-total did not differ between groups (t24=-1.671; p=0.108). **Amphetamine-induced rotation.** A mixed-model ANOVA revealed a main effect of GROUP, wheras both the main effect of TIME and the GROUP×TIME interaction were not significant. The percentage of ipsilesional rotations was greater in MCAO, compared to sham for all but three time points (all p<0.05). **Serial order learning.** All indexes were analyzed using a mixed-model ANOVA. The percentage of correct sequences yielded a significant SESSION main effect ($F_{3,72}$=15.586; p<0.001) and a GROUP×SESSION interaction ($F_{3,72}$=9.109; p<0.001). Incorrect sequences analysis revealed a SEQUENCE main effect ($F_{2,216}$=1729.357; p<0.001), a SESSION main effect ($F_{3,216}$=8.479; p<0.001) and a SEQUENCE×SESSION interaction ($F_{6,216}$=14.285; p<0.001).
Only a SESSION main effect were identified for both the number of sequences performed ($F_{3,72}$=13.722; p<0.001) and the mean latency between lever-presses for correct sequences ($F_{3,72}$=13.919; p<0.001). The number of premature food-tray visits (i.e., food-tray visits before completion of a correct sequence) yielded a SESSION main effect ($F_{3,69}$=10.97; p<0.001) and a GROUP×SESSION interaction ($F_{3,69}$=9.156; p<0.001). **Histological analysis.** In the MCAO group, mean infarct volume was 31.204±3.213 mm³. In three mice from the MCAO group, NeuN immunoreactivity was only absent from the dorsal striatum; in the other eight MCAO mice, ischemic infarct extended to the adjacent cortex (primary and secondary sensory cortex, sensorimotor cortex, piriform cortex, insular cortex) and hippocampus.

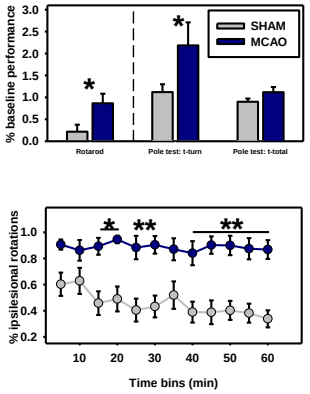


Figure 1. Top) Rotarod and vertical pole test performance. The MCAO group displayed poorer performance than the sham group on the rotarod, and took more time to turn over on a vertical pole. Bottom) Amphetamine-induced rotation test. MCAO mice showed a greater proportion of ipsilateral circlings. (*: p<0.05; **: p<0.01; ***:p<0.001)

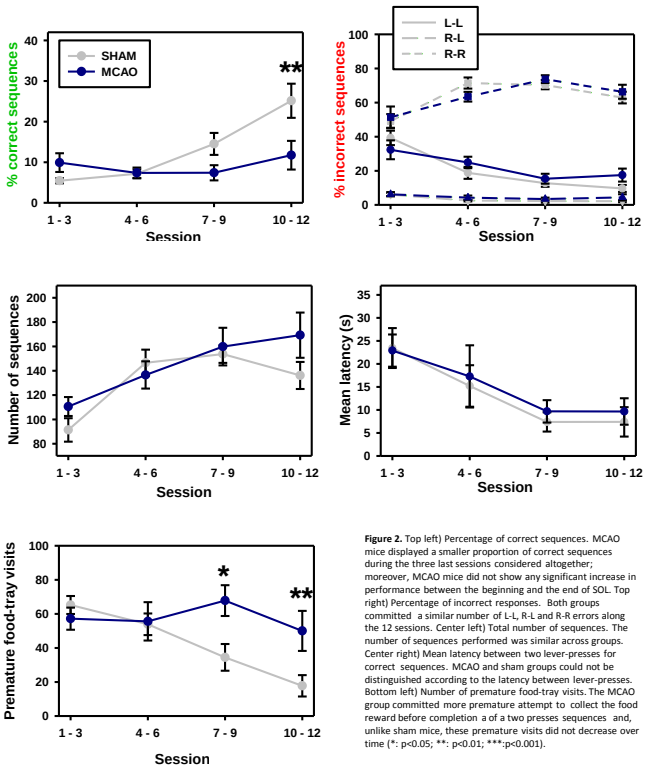


Figure 2. Top left) Percentage of correct sequences. MCAO mice displayed a smaller proportion of correct sequences during the three last sessions considered altogether; moreover, MCAO mice did not show any significant increase in performance between the beginning and the end of SOL. Top right) Percentage of incorrect responses. Both groups committed a similar number of L-L, R-L and R-R errors along the 12 sessions. Center left) Total number of sequences. The number of sequences performed was similar across groups. Center right) Mean latency between two lever-presses for correct sequences. MCAO and sham groups could not be distinguished according to the latency between lever-presses. Bottom left) Number of premature food-tray visits. The MCAO group committed more premature attempt to collect the food reward before completion a of a two presses sequences and, unlike sham mice, these premature visits did not decrease over time (*: p<0.05; **: p<0.01; ***:p<0.001).

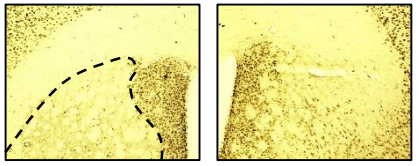


Figure 3. Microphotographs of ipsilateral (left panel) and controlateral (right panel) striatum 42 days after a 30 min MCAO (1:20). Decreased NeuN immunoreactivity revealed neuronal loss in the dorsolateral striatum (as delineated by the dotted line).

Discussion

Our results pointed to a deficit regarding procedural learning after a 30 min MCAO in mice, as well as an impaired motor coordination and an amphetamine-induced rotation bias.
Altered coordination in the rotarod and vertical pole test confirms that these tests seems reliable at identifying long-term motor impairment [3,4]
Similarly, **the amphetamine rotation test indicates clear differences between MCAO and sham groups**, which makes it useful for functional testing after cerebral ischemia in mice (as it has already been shown with rats [5]).
In the operant SOL test, we provide evidences that MCAO mice had difficulties to learn a simple sequences of lever-presses. This result does not seem to be consecutive to a greater number of errors, neither to an inability to perform lever-presses sequences. However, MCAO mice displayed a greater number of premature food-tray visits. This last index could point to an impairment to learn the proper behavioral sequence (i.e., left lever-press → right lever-press → food-tray visit).
Interestingly, habit formation is a form of S-R learning that heavily relies on dorsolateral striatum [2,6]. Histological analysis revealed that this structure was consistently infarcted after a 30 min MCAO. Thus, we suggest that **the infarction of dorsolateral striatum is accountable for the poor procedural performance in MCAO mice.**

1. Vakil, E., H. Blachstein, and N. Soroker, *Differential effect of right and left basal ganglionic infarctions on procedural learning.* Cogn Behav Neurol, 2004. **17**(2): p. 62-73.
2. Yin, H.H., *The sensorimotor striatum is necessary for serial order learning.* J Neurosci, 2010. **30**(44): p. 14719-23.
3. Balkaya, M., et al., *Characterization of long-term functional outcome in a murine model of mild brain ischemia.* J Neurosci Methods, 2013. **213**(2): p. 179-187.
4. Bouet, V., et al., *Sensorimotor and cognitive deficits after transient middle cerebral artery occlusion in the mouse.* Exp Neurol, 2007. **203**(2): p. 555-67.
5. Hudzik, T.J., et al., *Long-term functional end points following middle cerebral artery occlusion in the rat.* Pharmacol Biochem Behav, 2000. **65**(3): p. 553-62.
6. Yin, H.H., B.J. Knowlton, and B.W. Balleine, *Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning.* Behav Brain Res, 2006. **166**(2): p. 189-96.

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