

Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's dementia

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Abstract: Present study was designed to monitor the cognitive profile of the animals upon repeated administration of reserpine, so as to determine that whether these animals should be used as animal models of Parkinson's dementia. In the present study, reserpine was injected daily (once a day for three weeks) at the dose of 0.1mg/kg. Short- and long term memories were assessed using a Morris water maze, on weekly basis. Novel object recognition test was performed after completion of the treatment (day 21). Animals were decapitated on day 21 and brain samples were stored at -70°C until neurochemical analysis by HPLC-EC. Impairment of short- and long term activities (as monitored in Morris water maze) were not observed until after first week. Long term memory was found to be impaired earlier than the short term memory. Novel object recognition test also exhibited reserpine-induced impairment of working memory. Neurochemical analysis of the whole brain samples by HPLC-EC method showed that repeated administration of reserpine significantly increased DOPAC/ DA ratio ($p < 0.01$). While 5-HIAA/ 5-HT ratio was found to be decreased ($p < 0.05$) in reserpine injected animals. This further confirmed that these neurochemical deficits to be the underlying reason in memory impairment. In conclusion, present study provides evidence that repeated administration of reserpine can be used as a 'progressive' animal model of Parkinson's dementia. Results could be beneficial for face validity and screening of the drugs for the treatment of dementia secondary to Parkinson's and related disorders.

Keywords: Reserpine, Parkinson's dementia, Morris water maze, Novel object recognition test, open field

INTRODUCTION

One of the complications associated with Parkinson's Disease (PD) is dementia (Aarsland *et al.*, 2003). Dementia is characterized by a decline in cognitive faculties and occurrence of behavioral abnormalities which interfere with an individual's activities of daily living (Fadil *et al.*, 2009). Apart from the motor impairments, expressive cognitive deficits are also presented by parkinsonian patients (Shults, 2003). Such patients exhibit emotional deficits along with cognitive dysfunction (Fernandes *et al.*, 2008). Hoegh *et al.* (2013) have reported that approximately half of nursing home residents with PD may have Parkinson's dementia at any given time and they remain undiagnosed and largely undertreated. Besides, Fénelon *et al.* (2000) has reported 70% of demented PD patients being presented with visual hallucinations suggesting dementia to be an important risk factor for the development of hallucinations in these patients.

Parkinson's-associated emotional processing deficits were previously reported to be impaired by single administration of reserpine at the dose of 0.5mg/kg. However, they did not observe cognitive dysfunction after the same treatment (Fernandes *et al.*, 2008). To study the neurochemical and behavioral mechanisms involved in

the pathophysiology of PD associated cognitive deficits, animal models are widely used (Brooks and Dunnett, 2012). MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-lesioned rats are currently in use as animal models of the early phase of Parkinson's disease (Cunha *et al.*, 2001), but it is difficult to study the *progression* of Parkinson's as well as associated dementia, in these animal model.

Reserpine causes monoamine depletion (Ponzio *et al.*, 1984), which is similar to the monoamine depletion caused by the neuronal damages resulting from oxidative stress (Ebadi, *et al.*, 1996; Teixeira *et al.*, 2009). We do thereby suggest repeated reserpine administration as a progressive animal model of Parkinson's associated memory impairment/ dementia. However, reserpine induced Parkinson's dementia would have some limitations, as the drug does not induce the neuronal degeneration. Fernande *et al.* (2012) have reported impairment of motor activity as a result of monoamine-depleting effects of reserpine at the dose of 0.1mg/kg. However, they did not observed impairment of working memory at this dose.

The present study was therefore designed to study the effects of repeated administration of reserpine at the dose of 0.1 mg/kg, so that it could be used as an animal model of Parkinson's dementia, with progressive features. Others

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have reported Parkinsonian symptoms upon repeated administration (Fernandes *et al.*, 2008) of reserpine. However, they have observed no memory impairments (as evaluated by novel object recognition and discriminative avoidance tasks) in these animals. Silva *et al.* (2002) have reported the development of motor deficits upon single administration of reserpine at the dose of 0.1 mg/kg along with oral dyskinesia. We hereby, have repeatedly injected reserpine at the dose of 0.1 mg/kg, to evaluate its effects on memory. We also assessed exploratory activities of these animals in open field. Results could be beneficial in understanding the progressive pathophysiology of Parkinson's dementia and related disorders.

MATERIALS AND METHODS

Animals

Locally bred male Albino Wistar rats (weighing 180-220g; 8 weeks old) were purchased from HEJ Research Institute of Chemistry, Karachi and were housed individually under 12 hr light and dark cycles (lights on at 06:00 hr) and controlled room temperature ($24 \pm 2^\circ\text{C}$) with free access to tap water and cubes of standard rodent diet at least 7 days before starting the experiment so that they could become familiar to the environment. Animals were tested in light phase. Before starting the experiment, rats were accustomed to various handling procedures in order to nullify the psychological affliction of environment. All protocols for experimentation were approved and performed in strict accordance with the Institutional Animal Ethics Committee (IAEC).

Drugs and doses

Reserpine purchased from Sigma (St. Louis, MO) was used in the present study. Drug was freshly prepared before each experiment. Control animals were injected with saline (0.1ml/kg).

Experimental protocol

Twenty-four male rats were randomly assigned to two groups each containing twelve animals each: (i) saline (1.0ml/kg) and (ii) reserpine (0.1mg/kg) injected groups. Animals were injected with the saline or reserpine respectively for a period of three weeks (single injection/day). Basal activities of the animals were recorded in Morris water maze test before starting the experiment. Morris water maze test was performed on weekly basis, for assessing short- and long term memories. Novel object recognition task was performed on day 21.

Behavioral assessment

Monitoring Vacuous Chewing Movements

Vacuous chewing movements were recorded in the Skinner's box apparatus as described by Ikram *et al.*, (2007). Each burst of purposeless chewing movements, which remained continuous for at least 30 seconds, was counted as one. Recordings were made for 20 minutes.

Monitoring motor coordination

Procedure was same as described earlier (Ikram and Haleem, 2010). Rotorod apparatus was used for the determination of motor coordination. It consists of drums moving on a pulley. There is a timer at on base of the apparatus. Experiment was performed in quiet room. Animals were placed on the drums and as they started moving, the timer was started for recording the time. As soon as animal fell down, timer at the base was stopped and the time was recorded. Motor coordination was expressed as time in seconds for which animal maintained the grip on the moving drum.

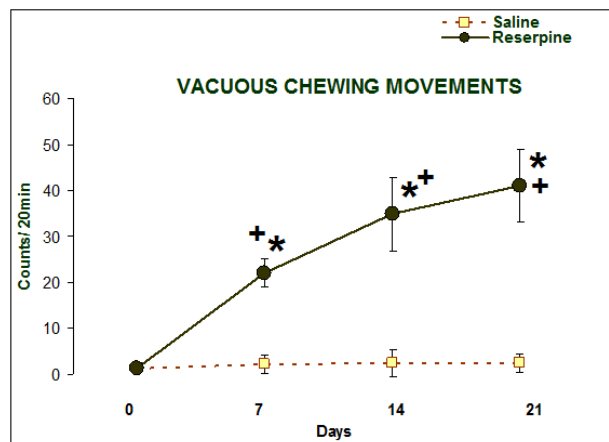


Fig. 1: Effect of repeated (once a day for 3 weeks) administration of reserpine (0.1 mg/kg) on vacuous chewing movements. Values are means $\pm$ S.D. (n=12). Significant differences by Newman-Keuls test: * $p < 0.01$ in repeated reserpine injected rats from their respective reserpine injected rats (day 0), following two-way ANOVA.

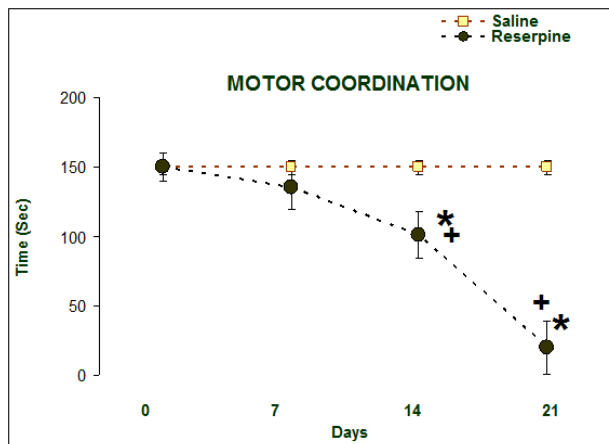


Fig. 2: Effect of repeated (once a day for 3 weeks) administration of reserpine (0.1mg/kg) on motor coordination. Values are means $\pm$ S.D. (n=12). Significant differences by Newman-Keuls test: * $p < 0.01$ in repeated reserpine injected rats from their respective repeatedly saline injected controls, + $p < 0.01$ from repeated reserpine injected rats (day 0), following two-way ANOVA.

Morris water maze test

The method for Morris Water Maze test was same as described elsewhere (Gordan *et al.*, 2012). Morris Water Maze test apparatus used in the present study consisted of a transparent rectangular glass tank (60 x 30cms) filled with room temperature-water opacified with powder milk, to the depth of 12cm. A wooden platform (15 x 13cms) was hidden 2cm below the surface of water in a fixed location. The experiment was performed after 30 minutes of injections. Initially the rats were trained and during the training session each rat was placed into the water facing the wall of the tank and allowed 120 seconds to locate and climb onto the submerged platform. The rat was allowed to stay on the platform for 10 seconds. If it failed to find the platform within the allowed time it was guided gently onto the platform. Memory functions of rats were tested by recording the retention latency (time taken by each rat to locate the hidden platform), 4hr post training (short term memory) and 24hr post training (long term memory). The cut off time for each session was 2 minutes.

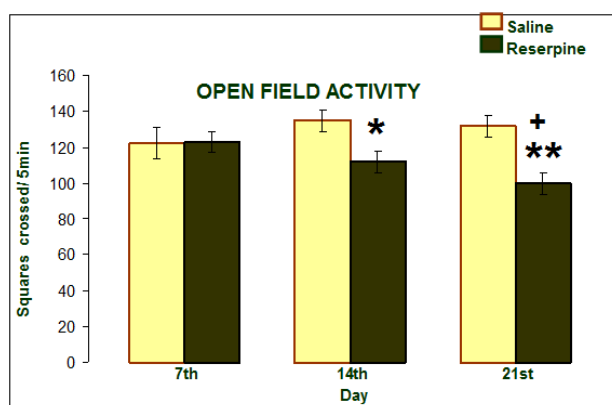


Fig. 3: Effect of repeated (once a day for 3 weeks) administration of reserpine (0.1 mg/kg) on weekly open field activity. Values are means $\pm$ S.D. (n=12). Significant differences by Newman-Keuls test: * $p < 0.05$, ** $p < 0.01$ in repeated reserpine injected rats from their respective repeatedly saline injected controls; + $p < 0.01$ from repeated reserpine injected rats (week 1), following two-way ANOVA.

Novel object recognition test

For the novel object recognition task the apparatus used was an open arena of (50 x 50) with 42 cm high walls. In order to saturate it with olfactory stimuli, cleaning of box was not allowed throughout the experiment. The objects to be discriminated were two glasses filled with white cement (used as familiar objects) in order to make them heavy enough so that rats could not be able to move them, and a metallic colored object (used as a novel object). The size of the objects was 2.5 times the size of the rat so that the rat could easily sniff it. During the first training session individual rat was permitted to explore the open

field arena for 10min, so that the animal was familiarized to the environment. After a delay of 24 hrs second training session was performed. During this session in the open field arena two similar novel objects were placed and allow the animal to explore them for 10 min. After the delay of 24hrs the retention test was performed in which the animal was positioned back into the similar environment the only difference is that one of the familiar objects (used in training session was now replaced by a novel object and each animal was given a maximum of 10 min to accumulate 30 seconds of object exploration (Antunes and Biala, 2012).

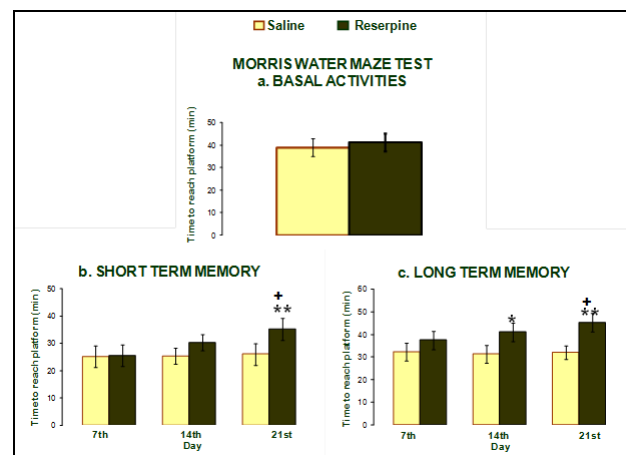


Fig. 4: Effects of repeated administration (once a day for 3 weeks) of reserpine (0.1 mg/kg) on basal Morris water maze activities. Values are means $\pm$ S.D. (n=12). a: Activities during training session. b: Weekly activities in Morris water maze, as monitored 4hr post injection (short term memory). c: Weekly activities in Morris water maze, as monitored 24hr post injection (long term memory). Significant differences by Newman-Keuls test: * $p < 0.05$, ** $p < 0.01$ in repeated reserpine injected rats from their respective repeatedly saline injected controls following two-way ANOVA.

Open field activity

A square area (76x76 cm) with walls 42 cm high was used to monitor activity in a novel environment. The floor of apparatus was divided by lines into 25 squares of equal size. Animals were injected with drug or saline and placed in the central square of the open field immediately after the injection. Numbers of squares crossed with all four paws were counted for 5 min (Ikram *et al.*, 2011; Ikram *et al.*, 2007).

Brain dissection

After decapitation, skull plates were cut and membrane covering the brain was removed with the help of fine forceps. Using spatula, brain was taken out and washed with ice-cold saline. The collected brains were immediately stored at -70°C for neurochemical estimations using High performance liquid

chromatography with electrochemical detection (HPLC-EC) (Beenish *et al.*, 2013).

Neurochemical estimations by HPLC-EC

HPLC-EC determination was carried out as described earlier (Ikram and Haleem, 2010). A 5 μ Shim-pack ODS separation column of 4.0mm internal diameter and 150mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1M phosphate buffer of pH 2.9 at an operating potential of 2000-3000 psi on Shimadzu HPLC pump. Electrochemical detection was achieved on Shimadzu LEC 6A detector at an operating potential of +0.8V.

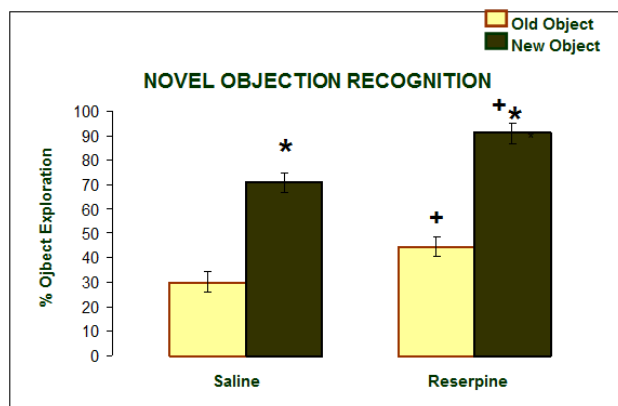


Fig. 5: Effects of repeated administration (once a day for 3 weeks) of reserpine (0.1 mg/kg) on novel object recognition test. Values are means $\pm$ S.D. (n=12). Significant differences by Newman-Keuls test: +p < 0.05 from their respective saline injected controls; *p < 0.05 from similarly injected rats, following two-way ANOVA.

STATISTICAL ANALYSIS

Results are represented as means $\pm$ SD. Statistical analysis was done by two-way analysis of variance (ANOVA) or Student's t-test (whichever applicable). Post hoc individual comparisons of groups were performed by Newman-Keuls test, following ANOVA. Values of p < 0.05 were considered significant.

RESULTS

Fig. 1 shows effects of repeated administration (once a day for 3 weeks) of reserpine (0.1 mg/kg) on vacuous chewing movements. Data analyzed by two-way ANOVA showed significant effects of reserpine (df= 1,88; F= 716.56; p<0.01), weekly monitoring (df= 3,88; F= 112.61; p<0.01) as well as interaction between the two (df= 3,88; F= 99.76; p<0.01). Post hoc analysis by Newman-Keuls test showed increased (p<0.01) vacuous chewing movements in reserpine injected rats on day 7, 14 and 21, as compared to their respective saline injected controls. The vacuous chewing movements in reserpine injected

rats on day 7, 14 and 21, were also comparable (p<0.01) with those of reserpine injected animals on day 0.

Fig. 2 shows effects of repeated administration (once a day for 3 weeks) of reserpine (0.1 mg/kg) on motor coordination. Data analyzed by two-way ANOVA showed significant effects of reserpine (df= 1,88; F= 1103.48; p<0.01), weekly monitoring (df= 3,88; F= 349.29; p<0.01) as well as interaction between the two (df= 3,88; F= 399.29; p<0.01). Post hoc analysis by Newman-Keuls test showed decreased (p<0.01) motor coordination in reserpine injected rats on day 14 and 21, as compared to their respective saline injected controls. The motor coordination of reserpine injected rats on day 14 and 21, was also comparable (p<0.01) with those of reserpine injected animals on day 0.

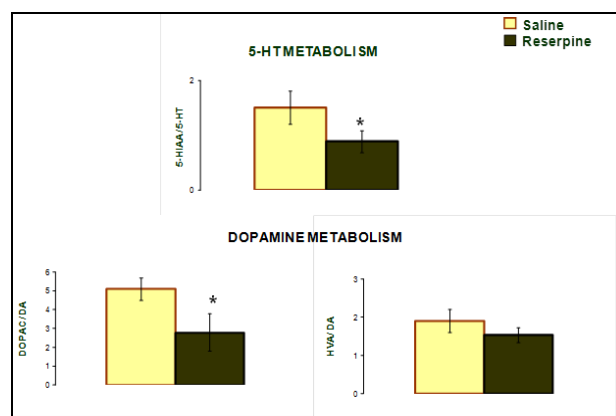


Fig. 6: Effect of repeated (once a day for 3 weeks) administration of reserpine (0.1mg/kg) on dopamine and 5-HT metabolism. Values are means $\pm$ S.D. (n=12). Significant differences by Student's t-test: *p<0.05, **p<0.01 in repeated reserpine injected rats from saline injected controls.

Fig. 3 shows effects of repeated administration (once a day for 3 weeks) of reserpine (0.1 mg/kg) on exploratory activity in open field apparatus. Data analyzed by two-way ANOVA showed significant effects of drug (df= 1,66; F= 115.23; p<0.01) but not those of weekly monitoring (df= 2,66; F= 2.24). However, interaction between the two (df= 1,66; F= 81.48; p<0.01) was found to be significant. Post hoc analysis by Newman-Keuls test showed decreased exploration of novel arena following second (p<0.05) as well as third (0.01) week. The decrease in reserpine injected animals following third week were comparable (p<0.01) with the same following first week.

Fig. 4 shows effects of repeated administration (once a day for 3 weeks) of reserpine (0.1 mg/kg) on Morris water maze test. Analysis of the data of basal activities (fig. 1a) by Student's t-test showed no significant difference between test and control groups. Effects of repeated administration (once a day for 3 weeks) of reserpine (0.1

mg/kg) on short term memory as monitored in Morris water maze on weekly basis, 4hr post injection (fig. 1b); as analyzed by two-way ANOVA showed significant effects of drug ($df= 1,66$; $F= 116.27$; $p<0.01$), weekly monitoring ($df= 1,44$; $F= 12.76$; $p<0.01$) as well as and interaction between the two ($df= 1,66$; $F= 20.11$; $p<0.01$). Post hoc analysis by Newman–Keuls test showed impairment ($p<0.01$) of short term memory in reserpine injected animals, after third week but not before it. Short term memory of reserpine injected animals was also found to be significantly greater ($p<0.01$) than the same after first week. Effects of repeated administration (once a day for 3 weeks) of reserpine (0.1 mg/kg) on short term memory as monitored in Morris water maze on weekly basis, 4hr post injection (fig. 1c); as analyzed by two-way ANOVA showed significant effects of drug ($df= 1,66$; $F= 107.51$; $p<0.01$), weekly monitoring ($df= 2,66$; $F= 8.75$; $p<0.01$) as well as interaction between the two ($df= 1,66$; $F= 8.75$; $p<0.01$). Post hoc analysis by Newman–Keuls test showed impairment of long term memory in reserpine injected animals, after second ($p<0.05$) as well as third week ($p<0.01$). Long term memory of reserpine injected animals was also found to be significantly greater ($p<0.01$) than the same after first week.

Fig. 5 shows effects of repeated administration (once a day for 3 weeks) of reserpine (0.1 mg/kg) on novel object recognition task. Data analyzed by two-way ANOVA showed significant effects of drug ($df= 1,44$; $F= 14.87$; $p<0.01$) as well as difference among the objects ($df= 1,44$; $F= 4.90$; $p<0.01$). However, interaction the two ($df= 1,44$; $F= 1.15$) was found to be nonsignificant. Post hoc analysis by Newman–Keuls test showed increased ($p<0.01$) % exploration of new object by saline as well as reserpine injected animals, as compared to old object. Reserpine injected animals exhibited increased ($p<0.01$) % exploration of both old as well as new object as compared to their respective saline injected controls.

Fig. 6 shows effects of repeated administration (once a day for 3 weeks) of reserpine (0.1 mg/kg) on dopamine and 5-HT metabolism. Data analysis by Student's t-test showed significant increase ($df= 22$; $t= 20.55$; $p<0.01$) in DOPAC/ DA ratio, with no effect on HVA/ DA ratio ($df= 22$; $t= 0.48$) upon repeated administration of reserpine. While, 5-HIAA/ 5-HT ratio was found to be decreased ($df= 22$; $t= 2.60$; $p<0.05$) in reserpine injected animals as compared to saline injected controls.

DISCUSSION

In the present study we observed progressive vacuous chewing movements (fig. 1) along with motor impairment (fig. 2). Motor responses were also evaluated as exploratory activity in an open field (fig. 3). Bradykinesia, tremor and rigidity are the behavioral

disorders featuring complex condition of PD (Valls-Sole and Valldeoriola, 2002). Vacuous chewing movements exhibited by the experimental animals mimic tardive dyskinesia associated with Parkinson's and related disorders (Thomas and Beal, 2007; Ikram *et al.*, 2007). Akinesia and rigidity (motor impairment) are Parkinsonian signs exhibited by rodents as a result of dopamine hypofunction. Impairment of motor coordination is an important parameter to detect the PD-associated symptoms in animal models (Diaz *et al.*, 2001). In rodents, these symptoms can be induced by direct dopamine antagonists like haloperidol (Ikram *et al.*, 2007) or by the neurotoxin 6-OHDA (Diaz *et al.*, 2001). Reserpine can also produce same effects by depleting vesicular monoamine storage pool.

Results from the present study revealed that repeated administration of reserpine at a dose of 0.1 mg/kg, impaired recognition memory as monitored in Morris water maze- and novel object recognition test. The preference for exploring new object was shown by reserpine injected animals (fig. 5), indicating that repeated reserpine administration impaired this memory in animals. Others also have reported reserpine-induced memory impairment. However, they have reported such memory impairing effects of reserpine after single administration of the drug (Fernandes *et al.*, 2008). However, we monitored a 'progressive' animal model of memory impairment and although novel object recognition test was performed at the end of the study, we monitored Morris water maze test on weekly basis, to monitor the 'progression' of the memory impairment.

In comparison with others, we have used relatively low dose of reserpine which does not usually produce an impairment of memory upon acute administration (Fernandes *et al.*, 2008). We also did not found any effect of reserpine injections on the memory of animals as assessed on day 7. An interesting finding of the present study was, that in Morris water maze test, short term memory was impaired after long term memory (fig. 4), suggesting an earlier loss of long term memory followed by short term memory loss. On day 14, long term memory was found to be impaired only without any impairment of short term memory. However, impairment of both short- and long term activities was observed on day 21. Cammarota *et al* (2005) have also reported that short term memory is more resistant to extinction, as compared to long term memory. However, both of these forms of memories are linked not only by mechanisms in the hippocampus that require NMDA receptors and protein synthesis, but also by other mechanisms requiring protein synthesis in the prefrontal cortex (Santini *et al.*, 2004). A comparison of short- and long term memories indicated that long term memories were impaired earlier than short term memories. Long term memories of both saline and

reserpine injected rats were comparable to short term memories of the same.

In present study, we did not find memory impairment until day 7. Thus, we hypothesize a progressive effect of the reserpine, resulting in memory impairment. Although reserpine is not inducing neuronal depletion (McCarty, 2006), it is producing memory impairment by producing monoamine depletion which is the final common feature in the naturally progressing dementia. Although very unlikely, a possibility of the context-dependent memory impairment (if any), just like any other behavioral sensitization (Ikram and Haleem, 2011), or context-dependent learning and memory, could not be ruled out. As the dopaminergic levels are important in mediating behavioral sensitization (Ikram *et al.*, 2012), others also have reported context-dependent potentiation of memory deficits by the blockade of dopaminergic neurotransmission (Schwartz *et al.*, 2003). Just to rule out this possibility, we also performed the novel objection recognition test at the end of the experiment, to cross check the memory impairment, as the novel object recognition task for rodents is a non-spatial, non-aversive memory test (Ennaceur and Delacour, 1988).

Animals spent more time in exploring new object in the test session during novel object recognition task, due to the fact that they easily recognize a previously presented (old) object (fig. 5). Reserpine injected animals presented preference for the new object, indicating that it could not affect this type of memory. In addition to this, the test animals spent comparatively more time in recognizing the old object as compared to saline injected animals. Different classes of dopaminergic receptors exert distinct effects on recognition memory (Nagai *et al.*, 2007); an activation of D₁ receptors can enhance recognition memory consolidation. Importantly, pharmacological activation of D₁ receptors enhances novel object recognition memory even under conditions in which control rats show significant retention (de Lima *et al.*, 2011).

A focus of current research is, whether short term memory is merely a step towards long term memory, or both are separate entities (Izquierdo *et al.*, 2006). However, an important role of serotonergic system has been suggested in short- as well as long-term memory (Meneses, 1999; Meneses, 2007). 5-HT can affect the memory directly and/or indirectly by modulating neurotransmitters such as acetylcholine and glutamate (Hu *et al.*, 2007; Madjid *et al.*, 2006). Although it is possible to separately study short- and long term memory, both mostly do function in a serial manner (Meneses, 2007). Apart from serotonin, dopamine is also important for working memory functions; mainly within prefrontal cortex (PFC) and a depletion of dopamine in PFC induces severe impairment in classic working memory task (Schmeichel *et al.*, 2013).

Decreased 5-HT and dopamine metabolism was observed in the present study (fig. 6), confirming these neurochemical deficits to be the underlying reason in memory impairment.

CONCLUSION

In conclusion, repeated administration of reserpine at the dose of 0.1 mg/kg, produces memory impairment. However, in contrast to previously reports, we have used the low dose of reserpine to induce a model exhibiting progressive loss of memory. We also monitored progressive motor impairment. Thus, contrary to previously reported dissociation between the reserpine-induced cognitive deficits and motor impairment (Fernande *et al.*, 2012) after single injection of reserpine at low dose (0.1 mg/kg), we report an association between the two at the same dose, upon repeated monitoring. Future research evaluating the mechanism of progressive neuronal changes in the monoaminergic and/or other neurotransmitter systems, in this animal model would make the picture further clear. Results could be beneficial for the face-validity and testing of pharmacotherapeutic agents which may be beneficial in the treatment of Parkinson's dementia and related disorders.

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