

Effects of single administration of apomorphine on memory and monoamine metabolism: A dose related study

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Abstract: In the present study, we have monitored dose dependent effects of apomorphine on learning and memory. Behavioral sensitization and craving, which develop upon repeated treatment with dopamine receptor agonist apomorphine, are major limitations of the therapeutic use of apomorphine in Parkinson's patients. Effects of single (intraperitoneal) injection of apomorphine at different doses (i.e., 0.5, 1.0, & 2.0 mg/ml/kg) on locomotion in a familiar environment (Skinner's box) and memory in Morris water maze were investigated. Results show significantly enhanced activity in Skinner's box in a dose dependant manner. Low dose (0.5 mg/ml/kg) of apomorphine impaired both short- as well as long-term memory while both high and moderate doses of the drug (1.0, & 2.0 mg/ml/kg) enhanced the cognitive profile in rats. However, the memory-enhancing effects of apomorphine at moderate (1.0 mg/ml/kg) dose were more pronounced as compared to high (2.0 mg/ml/kg) dose of the drug. Rats were decapitated on day 2. Whole brains of rats were collected and stored at -70°C. Biogenic amines (i.e., 5-Hydroxytryptamine; 5-HT and dopamine) and metabolites (i.e., Dihydroxyphenylacetic acid; DOPAC, Homovanillic acid; HVA & 5-Hydroxyindoleacetic acid; 5HIAA) were estimated by reverse phase High Performance Liquid Chromatography with electrochemical detector (HPLC-EC). Both low (0.5mg/ml/kg) as well as moderate (1.0mg/ml/kg) dose of apomorphine increased levels of dopamine, DOPAC, HVA, 5-HT and 5-HIAA. Whereas, high (4.0 mg/kg) dose of apomorphine increased levels of dopamine, DOPAC and HVA, while decreased 5-HT and 5-HIAA levels. Results would be helpful in elucidating memory enhancing effects of apomorphine at different doses and its implication for extending therapeutics in cognitive disorders.

Key words: Apomorphine, Parkinson's, Morris water maze test, learning, memory.

INTRODUCTION

Dementia interferes with daily functioning because of several cognitive deficits and affects approximately 6.5% of people over the age of 65 (Matthews *et al.*, 2013). Behavioral and psychological symptoms reported in dementia, involve dopaminergic neurotransmission. These could also be important targets for the treatment of behavioral and psychological symptoms of dementia. Holmes *et al.* (2001) have reported that psychosis and aggression in Alzheimer's disease is mediated by dopamine receptor gene variation. Patients with dopamine receptor genes DRD1 polymorphism were reported to be more aggressive or experience hallucinations. Hallucinations are very common in Alzheimer's disease as well and are associated with institutionalization and mortality (Scarmeas *et al.*, 2005) but could pose challenges for clinicians because of the risks of prescribing neuroleptics to dementia patients (Coon *et al.*, 2014).

An important role of dopamine is reported in learning and memory (Trossbach *et al.*, 2014; Yan *et al.*, 2014; LaLumiere, 2014; Puig *et al.*, 2014; Furin *et al.*, 2014). Intranasal dopamine administration can improve attention

and working memory in the 8-arm Olton maze in an ADHD rat model (Ruocco *et al.*, 2014). Dopamine (DA) plays an important role in regulating motor and limbic functions. Dopamine also regulates cognitive brain functions. Studies have shown that changes in the cognitive functions in diseases like schizophrenia, ADHD and in the early stages of Parkinson's disease, involve dysfunction of dopaminergic neurotransmission. These cognitive deficits could be ameliorated by normalizing dopaminergic neurotransmission.

Apomorphine, being dopaminergic agonist (Wang *et al.*, 2007), could be used to improve cognitive performance. Zarrindast *et al.*, (2003) have reported significant increase in locomotor behavior in a dose-dependent manner, following subcutaneous injections of apomorphine (2–10 mg/kg). These hyperlocomotive effects of apomorphine are mediated by the stimulation of D₂ receptors because selective blockade of D₂ receptors suppressed apomorphine-induced motor behavior (Millan *et al.* 2004, Zvezdochkina *et al.*, 2006).

Apomorphine produces hyperlocomotive effects in a dose-dependant manner and could be used for the treatment of Parkinson's and related disorders (Ikram *et al.*, 2011; Ikram *et al.*, 2012; Ikram and Haleem, 2011).

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The present study was therefore designed to study the effects of various doses of apomorphine on learning and memory as monitored in Morris water maze and to select optimum dose of apomorphine, which could increase cognitive performance with a affecting dopamine levels.

MATERIALS AND METHODS

Animals

Experimental design was carried out in strict accordance with the guidelines by the Institutional Animal Ethics Committee (IAEC). Albino-Wistar rats (weighing 180-220 grams) provided by the HEJ Research Institute of Chemistry, University of Karachi were housed individually in Perspex cages. Rats were placed in an environmentally controlled room at room temperature ($25 \pm 2^\circ\text{C}$) under a 12:12 h light/ dark cycle (lights on at 6:00 hr). A three day acquisition phase was allowed before starting the experiments so that the rats could become familiar with the environment.

Drug and doses

Apomorphine-HCl (Sigma, St. Louis, USA) was dissolved in saline (0.9% NaCl) and injected intraperitoneally at the doses of 0.5, 1.0, and 2.0 mg/ml/kg to the respective rats. Drug was freshly prepared before starting the experiment. Saline (0.9% NaCl solution; 1ml/kg) was injected to control rats.

Experimental protocol

Rats were randomly assigned to four groups each containing six rats: (i) Saline-, (ii) Apomorphine (0.5 mg/ml/kg)-, (iii) Apomorphine (1.0 mg/ml/kg)-, and (iv) Apomorphine (2.0 mg/ml/kg)-injected rats. Rats were injected with saline (0.9% NaCl solution) or respective dose of apomorphine. Motor activities in Skinner's box were recorded 10min post injection. While short-term memory in the Morris water maze was tested 1hr- as well as 6hr post injection. Long term memory in the Morris water maze was tested 24hr post-injection. Food in take, and body weights were recorded and reported as cumulative food intake and %growth rates.

Behavioral procedures

Dose-dependent effect of apomorphine on motor activity in a familiar environment

15 min before injection, rats were transferred to the activity cages (transparent perspex cages with dimensions 26x26x26 cm) with saw dust covered floor. Activity was monitored as counts of cage crossings/10 min starting 5min post injections (Ikram *et al.*, 2007; Farhan *et al.*, 2014).

Morris water maze test

The procedure was essentially similar as described elsewhere (Ikram *et al.*, 2014; Mirza *et al.*, 2013). The water maze apparatus used in the present study consisted of a tank, 210cm in diameter and filled to a level that was

2cm higher than the platform height. Water temperature was at room temperature, $21 \pm 1^\circ\text{C}$. The platform (10 cm×10 cm) was made of clear acrylic and was hidden 2cm below the surface of water in a fixed location. Water was made opaque by adding milk to it. 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 second.

If it failed to find the platform within the allowed time it was guided gently onto the platform. Rats were tested for short-term and long-term memory by recording the retention latency (time taken by each rat to locate the hidden platform). The cut off time for each session was 5 minutes.

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 neuro chemical estimations using High performance liquid chromatography with electrochemical detection (HPLCEC) (Ikram *et al.*, 2012; Mirza *et al.*, 2013).

Neurochemical estimations by HPLC-EC

HPLC-EC determination was carried out as described earlier (Ikram *et al.* 2007, Ikram *et al.* 2011, Ikram *et al.* 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.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS (ver 17) software. Data were analyzed by one-way ANOVA. Post-hoc analysis by Tukey's test was performed to compare individual differences among the groups. Values of $p < 0.05$ were considered as significant.

RESULTS

fig. 1 shows effects of different doses of apomorphine on growth rates and food intakes. Data on growth rates (fig. 1a) as analyzed by one-way ANOVA showed significant effects of treatment ($F_{3,20} = 4.98$, $P < 0.05$). Post hoc analysis by Tukey's test showed that apomorphine at all three doses decreased ($P < 0.01$) food intake as compared

to saline injected rats. fig. 1b shows effects of different doses of apomorphine on food intake. Analysis of the data by one-way ANOVA showed significant effects of treatment ($F_{3,20} = 11.93$, $P < 0.01$). Post hoc analysis by Tukey's test showed that apomorphine at moderate and high dose (i.e., 1.0- and 2.0 mg/ml/kg) decreased ($P < 0.05$) food intake as compared to saline injected rats.

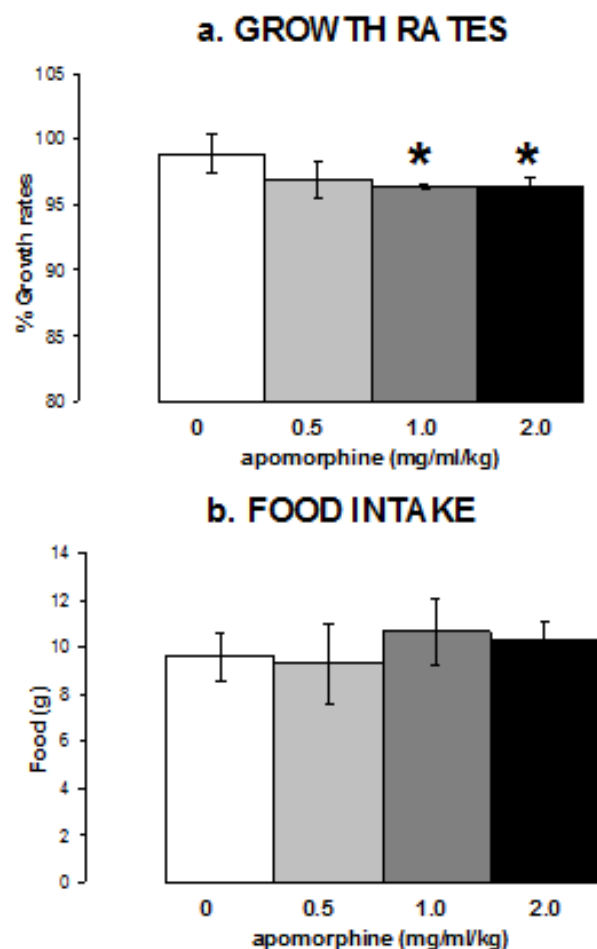


Fig. 1: Effects of apomorphine at three different doses (0.5, 1.0 and 2.0 mg/ml/kg) on growth rates, as monitored 24hr post injection. Significant differences by Tukey's test: * $P < 0.05$ as compared to saline injected controls following one-way ANOVA.

DISCUSSION

In the present study, hypophagic effects of apomorphine were observed following first injection of the drug at moderate and high dose (1.0 and 2.0 mg/ml/kg). While no effect on feeding behavior was observed at low dose (0.5 mg/ml/kg) of apomorphine. Stimulation of presynaptic dopaminergic receptors at low (0.2 mg/ml/kg) doses of apomorphine can inhibit the dopaminergic neurotransmission (Benoit-Marand *et al.*, 2001; Phillips *et al.*, 2002). Dopaminergic systems are known to influence reward-based feeding (Berthoud, 2007). Billes *et al.* (2012) have reported that mesolimbic dopamine systems

importantly dopamine D_2 receptors are important for homeostatic regulation of feeding. Blockade of D_2 receptors/ unavailability of dopamine at these receptors would impair feeding signals.

In the present study, hypophagic effects monitored after single injection of apomorphine at moderate and high doses (1.0- and 2.0mg/ml/kg) could be explained in terms of increased dopaminergic neurotransmission. Since, apomorphine at high dose (2.0mg/ml/kg) has been reported to increase dopamine metabolism (Ikram *et al.* 2011), an increased availability of dopamine in reward center may alleviate the rewarding effects of natural reinforcers like food.

In the present study, short term memory monitored in MWM (1hr post injection) was found to be improved at the moderate dose (1.0mg/ml/kg) of apomorphine. However, at low dose (0.5mg/ml/kg), impairment in memory was monitored while no effect was observed at high dose of apomorphine.

Likewise, short term memory following 6hr post injection impairs memory at low and high doses (0.5 and 2.0mg/ml/kg). Gourgiotis *et al.* (2012) have reported that apomorphine at the dose of 0.5mg/ml/kg disrupt memory in NORT (Novel object recognition test). Systemic injection of apomorphine (0.05 or 0.5 mg/ml/kg) produce differential impairing effects on short- and long term retention of an inhibitory avoidance task (Picada *et al.*, 2002). This disruption in memory at low dose (0.5mg/ml/kg) might be due to the stimulation of presynaptic receptors which result in decreased dopaminergic neurotransmission.

Intranasal dopamine could be beneficial for improving attention and working memory in the 8-arm Olton maze in an ADHD rat model (Ruocco *et al.*, 2014). These cognitive-enhancing effects of intranasal dopamine, along with evidence for anti-depressant and anti-parkinsonian actions can bolster the prospect of considering intranasal dopamine application as a therapeutic measure against cognitive and mood-related deficits (Trossbach *et al.*, 2014).

In the present study, long term memory was found to be increased at moderate and high dose (1.0 and 2.0mg/ml/kg) of apomorphine following 24hr post injection. Nagai *et al.* (2007) have reported that extracellular signal-regulated kinase1/2 (ERK1/2) and dopaminergic system is involved in learning and memory in prefrontal cortex region (PFC). Exposure to novel object increase the amount of phosphorylated ERK1/2 and dopamine $D1$ receptor agonist increases the phosphorylation of ERK1/2 in the PFC region. Long term memory can be impaired by the inhibition of ERK kinase 24 h after the training session. However, inhibition did not produce any effect on short term memory.

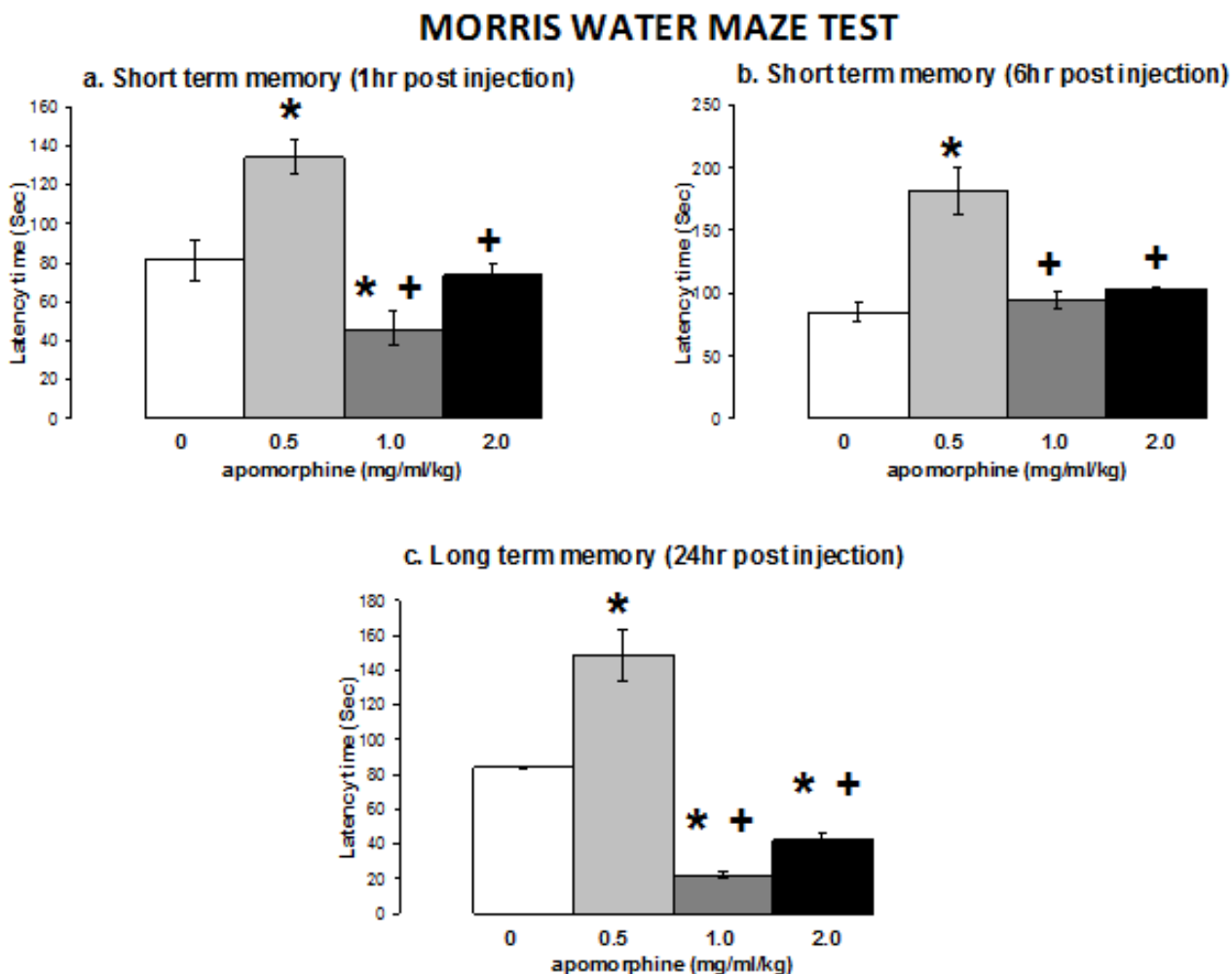


Fig. 2: Effects of apomorphine at three different doses (0.5, 1.0 and 2.0 mg/ml/kg) on long term memory as monitored 24hr post injection in Morris water maze. Significant differences by Tukey's test: * $P < 0.01$ as compared to saline injected controls, + $P < 0.01$ as compared to low dose (0.5mg/ml/kg) injected rats following one-way ANOVA.

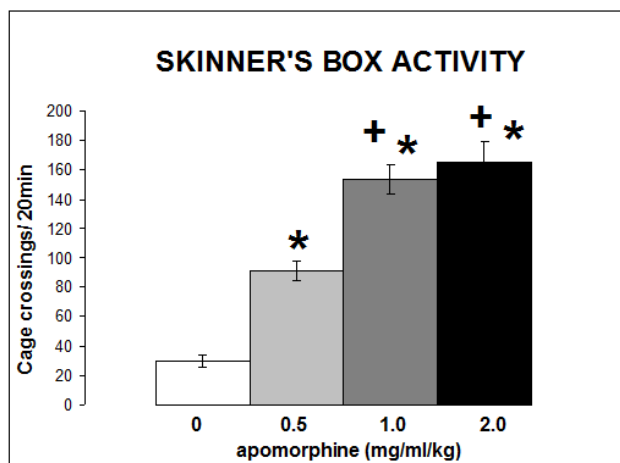


Fig. 3: Effects of apomorphine at three different doses (0.5, 1.0 and 2.0 mg/ml/kg) on locomotive activity as monitored post first injection in Skinner's box. Significant differences by Tukey's test: * $P < 0.01$ as compared to saline injected controls, + $P < 0.01$ as compared to low dose (0.5mg/ml/kg) injected rats following one-way ANOVA.

Apomorphine increases the locomotor activity (Bloise *et al.*, 2007). Ikram *et al.* (2011) have also reported earlier that apomorphine increases locomotor activity in a dose dependent manner in a familiar environment of skinner's box. Corollary results found in our laboratory by increasing the dose of apomorphine from 0.5 to 2.0mg/ml/kg increase locomotor activity. Apomorphine at the dose of 1.0mg/ml/kg can increase the locomotor activity without disturbing dopamine metabolism.

CONCLUSION

The objective of present study was to select minimum dose of apomorphine, which could increase cognitive performance. Results have shown that moderate (1.0mg/ml/kg) dose of apomorphine is found to be the optimum dose, which could increase the cognitive performance. Therefore, this dose of apomorphine could be used for the treatment of learning and memory disorders like Alzheimer's disease, ADHD etc. However, the use of apomorphine is associated with the addiction

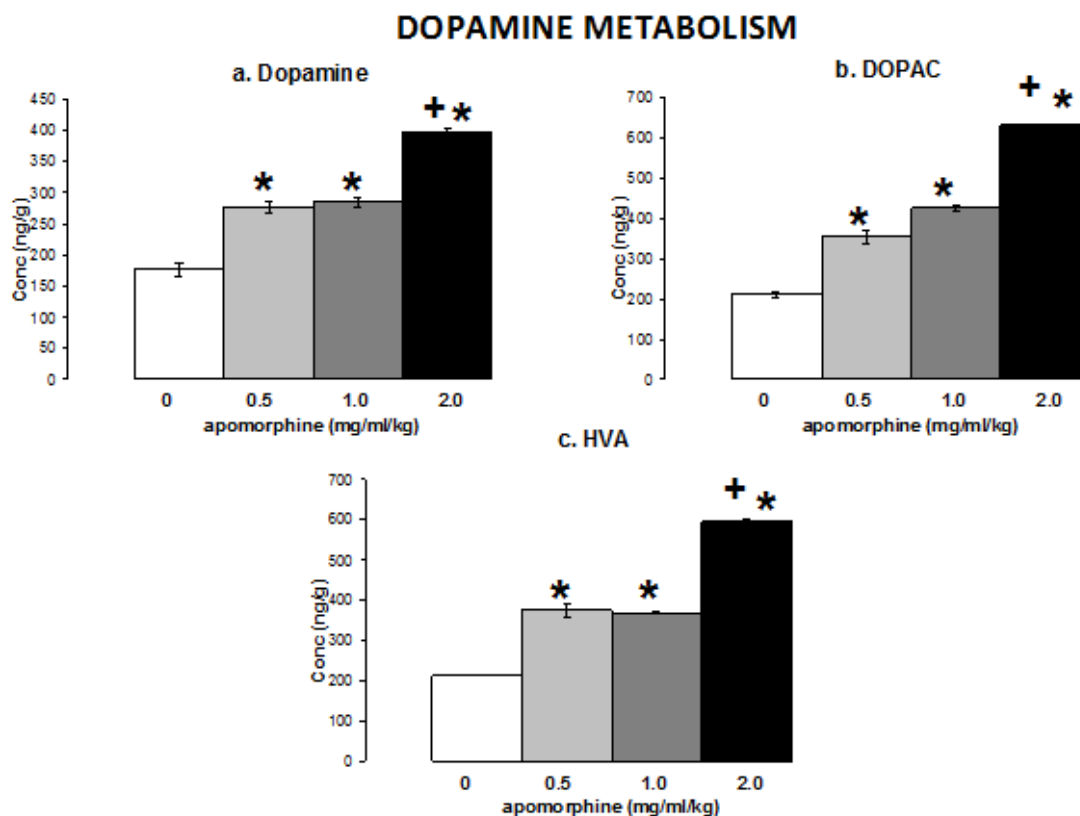


Fig. 4: Effects of different doses of apomorphine on dopamine metabolism. Significant differences by Tukey's test: * $P < 0.01$ as compared to saline injected controls, + $P < 0.01$ as compared to low dose (0.5 mg/ml/kg) injected rats, following one-way ANOVA

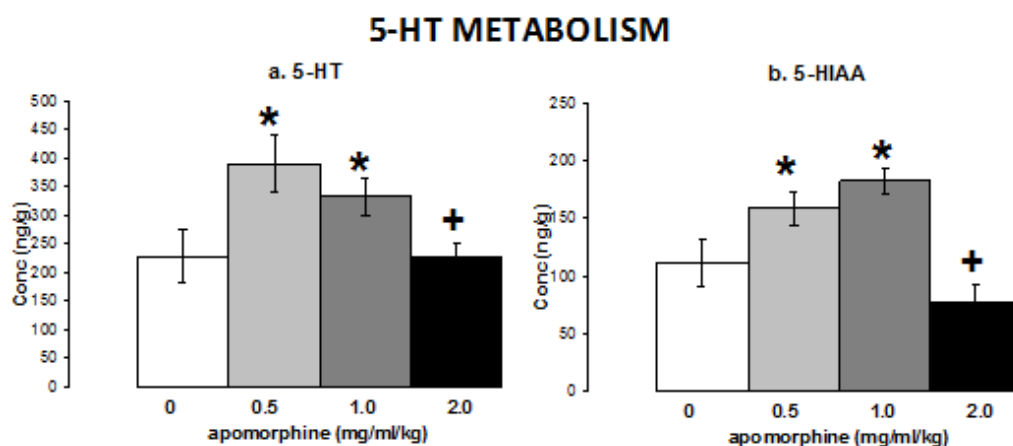


Fig. 5: Effects of different doses of apomorphine on 5-HT metabolism. Significant differences by Tukey's test: * $P < 0.01$ as compared to saline injected controls, + $P < 0.01$ as compared to low dose (0.5 mg/ml/kg) injected rats, following one-way ANOVA

therefore further study is required to enhance the therapeutic potential of apomorphine by ameliorating the addictive properties of apomorphine.

REFERENCES

- Benoit-Marand M, Borrelli E and Gonon F (2001). Inhibition of dopamine release via presynaptic D2 receptors: time course and functional characteristics in vivo. *J. Neurosci.*, **21**: 9134-9141.
- Berthoud HR (2007). Review Interactions between the "cognitive" and "metabolic" brain in the control of food intake. *Physiol. Behav.*, **91**: 486-498.
- Billes SK, Simonds SE and Cowley MA (2012). Leptin reduces food intake via a dopamine D2 receptor-dependent mechanism. *Mol. Metab.*, **1**: 86-93.

- Bloise E, Carey RJ and Carrera MP (2007). Behavioral sensitization produced by a single administration of apomorphine; implications for the role of pavlovian conditioning in the mediation of context-specific sensitization. *Pharmacol. Biochem. Behav.*, **86**: 449-457.
- Collins GT, Jackson JA, Koek W and France CP (2014). Effects of dopamine D2-like receptor agonists in mice trained to discriminate cocaine from saline: Influence of feeding condition. *Eur. J. Pharmacol.*, **729**: 123-131.
- Coon JT, Abbott R, Rogers M, Whear R, Pearson S, Lang I, Cartmell N and Stein K (2014). Interventions to Reduce Inappropriate Prescribing of Antipsychotic Medications in People With Dementia Resident in Care Homes: A Systemic Review. *J. Am. Med. Doc. Ass.*, **15**: 706-718.
- Farhan M, Ikram H, Kanwal S and Haleem DJ (2014). Unpredictable Chronic Mild Stress induced Behavioral Deficits: A Comparative Study in Male and Female Rats. *Pak. J. Pharm. Sci.*, **27**: 879-884.
- Furini JC, Myskiw BE, Schmidt LA, Marcondes I and Izquierdo (2014). D1 and D5 dopamine receptors participate on the consolidation of two different memories. *Behav. Brain. Res.*, **271**: 212-217.
- Gourgiotis I, Kampouri NG, Koulouri V, Lempesis IG, Prasinou MD, Georgiadou G and Pitsikas N (2012). Nitric oxide modulates apomorphine-induced recognition memory deficits in rats. *Pharmacol. Biochem. Behav.*, **102**: 507-514.
- Haleem DJ, Ikram H, Haider S, Parveen T and Haleem MA (2013). Enhancement and inhibition of apomorphine-induced sensitization in rats exposed to immobilization stress: Relationship with adaptation to stress. *Pharmacol. Biochem. Behav.*, **112**: 22-28.
- Haleem DJ, Ikram H and Haleem MA (2014). Inhibition of apomorphine-induced conditioned place preference in rats co-injected with buspirone: Relationship with serotonin and dopamine in the striatum. *Brain Res.*, **1586**: 73-82.
- Holmes C, Smith H, Ganderton R, Arranz M, Collier D, Powell J and Lovestone S (2001). Psychosis and aggression in Alzheimer's disease: The effect of dopamine receptor gene variation. *J. Neurol. Neurosurg. Psychiatr.*, **71**: 777-779.
- Ikram H, Ahmed S and Haleem DJ (2011). Effects of Apomorphine on Locomotor Activity and Monoamine Metabolism: A Dose Related Study. *Pak. J. Pharm. Sci.*, **24**: 315-321.
- Ikram H, Choudhary AM and Haleem DJ (2012). Regional Neurochemical Profile following Development of Apomorphine-induced Reinforcement. *Pak. J. Pharm. Sci.*, **25**: 513-519.
- Ikram H and Haleem DJ (2011). Attenuation of Apomorphine-Induced Sensitization by Buspirone. *Pharmacol. Biochem. Behav.*, **99**: 444-450.
- Ikram H, Mushtaq F and Haleem DJ (2014). Dose-Dependent Effects of Tryptophan on Learning and Memory. *Pak. J. Pharm. Sci.*, **27**: 1131-1135.
- Ikram H, Samad N and Haleem DJ (2007). Neurochemical and Behavioral Effects of m-CPP in a Rat Model of Tardive Dyskinesia. *Pak. J. Pharm. Sci.*, **20**: 188-195.
- Matthews FE, Arthur A, Barnes LE, Bond J, Jagger C, Robinson L and Brayne C (2013). A two-decade comparison of prevalence of dementia in individuals aged 65 years and older from three geographical areas of England: Results of the cognitive function and ageing study I and II. *The Lancet*, **382**: 1405-1412.
- Millan MJ, Seguin L, Gobert A, Cussac D and Brocco M (2004). The role of dopamine D3 compared with D2 receptors in the control of locomotor activity: A combined behavioral and neurochemical analysis with novel, selective intake antagonists in rats. *Psychopharmacol.*, **174**: 341-357.
- Mirza B, Ikram H, Bilgrami S, Haleem DJ and Haleem MA (2013). Neurochemical and behavioral effects of green tea (*Camellia sinensis*): A model study. *Pak. J. Pharm. Sci.*, **26**: 511-516.
- Nagai T, Takuma K, Kamei H, Ito Y, Nakamichi N, Ibi D, Nakanishi Y, Murai M, Mizoguchi H, Nabeshima T and Yamada K (2007). Dopamine D1 receptors regulate protein synthesis-dependent long-term recognition memory via extra cellular signal-regulated kinase 1/2 in the prefrontal cortex. *Learn Mem.*, **14**: 117-125.
- Phillips PE, Hancock PA and Stamford JA (2002). Time window of auto receptor-mediated inhibition of limbic and striatal dopamine release. *Synapse*, **44**: 15-22.
- Picada JN, Schroder N, Izquierdo, Henriques JA and Roesler R (2002). Differential neurobehavioral deficits induced by apomorphine and its oxidation product, 8-oxo apomorphine-semiquinone, in rats. *Eur. J. Pharmacol.*, **443**: 105-111.
- Puig MV, Antzoulatos EG and Miller EK (2014). Prefrontal dopamine in associative learning and memory. *Neuroscience*, **282**: 217-229.
- Ruocco LA, Treno C, Gironi Carnevale UA, Arra C, Mattern C, Huston JP, de Souza Silva MA, Nikolaus S, Scorziello A, Nieddu M, Boatto G, Illiano P, Pagano C, Tino A and Sadile AG (2014). Prepuberal intranasal dopamine treatment in an animal model of ADHD ameliorates deficient spatial attention, working memory, amino acid transmitters and synaptic markers in prefrontal cortex, ventral and dorsal striatum. *Amino Acids*, **46**: 2105-2122.
- Ruocco LA, Treno C, Gironi Carnevale UA, Arra C, Mattern C, Huston JP, de Souza Silva MA, Nikolaus S, Scorziello A, Nieddu M, Boatto G, Illiano P, Pagano C, Tino A and Sadile AG (2014). Prepuberal intranasal dopamine treatment in an animal model of ADHD ameliorates deficient spatial attention, working memory, amino acid transmitters and synaptic markers

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- Scarmeas N, Brandt J, Albert M, Hadjigeorgiou G, Papadimitriou A, Dubois B, Sarazin M, Devanand D, Honig L, Marder K, Bell K, Wegesin D, Blacker D and Stern Y (2005). Delusions and hallucinations are associated with worse outcome in Alzheimer disease. *Arch. Neurol.*, **62**: 1601-1608.
- Trossbach SV, de Souza Silva MA, Huston JP, Korth C and Mattern C (2014). Intranasal dopamine treatment reinstates object-place memory in aged rats. *Neurobiol. Lear. Mem.*, **114**: 231-235.
- Wang WF, Lei YP, Tsenq T, Hsu WY, Wang CF, Hsu CC and Ho YJ (2007). Effects of apomorphine on the expression of learned helplessness behavior. *Chin. J. Physiol.*, **50**: 63-68.
- Yan Y, Newman AH and Xu M (2014). Dopamine D1 and D3 receptors mediate reconsolidation of cocaine memories in mouse models of drug self-administration. *Neuroscience*, **278**: 154-164.
- Zarrindast M-R, Gholami A, Sahraei H and Haeiri-Rohani A (2003). Role of nitric oxide in the acquisition and expression of apomorphine- or morphine-induced locomotor sensitization. *Eur. J. Pharmacol.*, **482**: 205-213.
- Zvezdochkina NV, Muranova LN, Andrianov VV, Archipova SS, Gainutdinovkh L, Golubev AI and Pleshchinskii IN (2006). Locomotor responses and neuron excitability in conditions of haloperidol blockade of dopamine receptors in vertebrates and invertebrates. *Neurosci. Behav. Physiol.*, **36**: 21-27.