**Function of opioidergic and dopaminergic antagonists on both spatial and object novelty
detection deficits induced in rodent model of hepatic encephalopathy**

Mohammad Nasehi^{a*}, Fatemeh Mafi^b, Mohaddeseh Ebrahimi-Ghiri^c, Mohammad-Reza
Zarrindast^{d,e,f,g,h*}

^a Cognitive and Neuroscience Research Center (CNRC), Tehran Medical Sciences Branch,
Islamic Azad University, Tehran, Iran

^b Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical
Sciences, Kerman, Iran

^c Department of Biology, Faculty of Sciences, University of Zanjan, Zanjan, Iran

^d Department of Pharmacology School of Medicine, Tehran University of Medical Sciences,
Tehran, Iran

^e Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran,
Iran

^f School of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran,
Iran

^g Medical Genomics Research Center, Tehran Medical Sciences Branch, Islamic Azad University,
Tehran, Iran

^h Institute for Cognitive Science Studies (ICSS), Tehran, Iran

*Corresponding authors: M. Nasehi (Nasehi@iricss.org; Cognitive and Neuroscience Research Center (CNRC), Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran) and M.R. Zarrindast (zarinmr@ams.ac.ir; Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran).

P.O.Box 13145-784

Tel: +9821-66402569

Fax: +9821-66402569

Abstract

Liver disease has been known for a long time to affect brain function. We now report the function of opioidergic and dopaminergic antagonists on both spatial and object novelty detection deficits induced by hepatic encephalopathy (HE) following bile duct ligation (BDL), a model of chronic liver disease. Assessment of spatial and object novelty detection memories was carried out in the non-associative task. It consists of placing mice in an open field containing five objects and, after three sessions of habituation, examining their reactivity to object displacement (spatial novelty) and object substitution (object novelty). Both spatial and object novelty detection memories were impaired by BDL after 4 weeks. In the BDL mice, pre-test intraperitoneal administration of naloxone (μ -opioidergic receptor antagonist) at dose of 0.9 mg/kg restored while sulpiride (D2-like dopamine receptor antagonist) at dose of 40 mg/kg potentiated object novelty detection memory deficit. However, SCH23390 (D1-like dopamine receptor antagonist) at dose of 0.04 mg/kg or sulpiride (20 mg/kg) restored spatial novelty detection memory deficit. Moreover, SCH23390 or sulpiride impaired while naloxone did not alter both memories in sham-operated mice. Furthermore, subthreshold dose co-administration of dopaminergic antagonists together or each one plus naloxone did not alter both memory impairments in BDL mice, while all of three co-administration groups impaired object novelty detection and co-administration of naloxone plus sulpiride impaired spatial detection memory in sham-operated mice. In conclusion, we suggest that opioidergic and dopaminergic systems through separate pathways may contribute in memory impairments induced by BDL in the non-associative task.

Keywords: Bile duct ligation; Dopamine; Opioid; Spatial and object novelty detection

1. Introduction

Alteration of normal brain function is a characteristic complication of both acute and chronic liver failures and are known as hepatic encephalopathy (HE) [1]. Bile duct ligation (BDL), according to the International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN) is a common model for chronic liver failure which reflects HE associated with cirrhosis and portal hypertension [2]. HE is characterized by deficits in all neurotransmitter systems in the brain. In particular, significant alterations in glutamatergic and monoaminergic mechanisms, as well as endogenous opioid neurotransmitter, γ -amino butyric acid (GABA), serotonergic, cholinergic and dopaminergic systems have been reported in the HE animal model [1, 3-6]. Cognitive function is also altered in rats and mice with BDL model of HE [7]. It has been reported that the expression of several factors involved in learning and memory changes in hippocampus of BDL-induced HE [6].

Dopamine (DA), a prominent neuromodulator, is implicated in many neuropsychiatric disorders. It has wide-ranging effects on both cortical and subcortical brain regions and on many types of cognitive tasks that rely on a variety of different learning and memory systems [8]. Five DA receptor subtypes (D1–D5) are grouped into two families on the basis of structural homology and biochemical characteristics. The family of D1-like receptors includes the D1 and D5 subtypes, and the family of D2-like receptors includes the D2, D3, and D4 subtypes. D1-like receptors are expressed throughout the brain, while D2-like receptors exhibit more regional specificity [9]. These differences have important consequences for cognition and behavior [10, 11]. All of DA receptors are slow-acting metabotropic receptors that are linked to G proteins. Activation of D1 receptors leads to an increase in intracellular levels of cAMP, whereas activation of D2 receptors leads to decreases in the levels of intracellular cAMP. In addition, phasic versus tonic DA release may have differential effects on D1 and D2 receptors [12]. It has been known that DA has two prominent neuromodulatory effects: a fast effect in which the signal-to-noise

ratio of the postsynaptic glutamate response is increased [13, 14], and a slower effect in which synaptic plasticity is potentiated [15, 16]. A decrease has been observed in the DA levels in cortex, hippocampus, striatum and cerebellum along with an increase in the activity of monoamine oxidase B (MAO-B) enzyme following BDL surgery indicating alterations in the dopaminergic system [6]. In addition, BDL rats display a diminution in striatal DA concentration similar to that observed in cirrhotic patients [17]. The concentration of DA metabolite, homovanillic acid, has been shown to be increased in HE patients along with the activity of DA metabolizing enzyme MAO-A and -B as a result of chronic hyperammonemia [18].

In addition, our previous findings showed that there is a synergistic effect between hippocampus mu opioid and DA D1-like receptors in memory impairment of BDL mice [19]. It has been well known that opioidergic system and its receptors, especially mu opioid receptor, is involved in cognitive functions such as learning and memory and altered in BDL models [20]. In view of the link between opioid or dopaminergic system and BDL, and the involvement of these systems in learning and memory processes, the present study was designed to assessment the function of opioidergic and dopaminergic antagonists on spatial and object novelty detection memory deficits induced in BDL mice in the non-associative task.

2. Material and methods

2.1. Animals

Male NMRI mice (30-35 g) bred at the Institute for Cognitive Science Studies (ICSS), Tehran, Iran. Animals were housed in an animal room that was maintained at $22 \pm 2^{\circ}\text{C}$ with a 12-h light: 12-h dark cycle. Food and water were available *ad libitum*. All the protocols in this study were approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran.

2.2. Surgery

There were two experimental groups: sham-operated and BDL mice. Laparotomy was performed under general anesthesia induced by an intraperitoneal (i.p.) injection of ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg). The sham-operation consisted of a laparotomy and bile duct identification and manipulation without ligation or resection. In the BDL group, the main bile duct was first ligated using two ligatures approximately 0.5 cm apart and then transected at the midpoint between the two ligatures. Immediately after the operation each animal was placed alone in a cage to prevent wound dehiscence and then moved to its original cage 4 h after surgery [19, 21, 22]. Operative mortality was less than 5%.

2.3. Reaction to novelty apparatus

The apparatus was based on that used by [23] with a minor modification in objects [24, 25]. It was a metal circular box, 60 cm in diameter with a 20 cm-high wall, and a floor painted white and divided into 16 identical sectors by black lines. The wall of the arena was white, with a black striped pattern (1.5 cm thick line), 20 cm wide and 10 cm high. The apparatus was illuminated by a red light (80 W) located on the ceiling. A video camera above the field was connected to a video recorder and a monitor. Six objects were present in the circular box: A cube (a metal-plated parallelepiped, measuring 7 cm × 4 cm × 4 cm with irregular holes distributed on all sides), a cone (a plastic cone on a transparent cylinder base, 8 cm in diameter and 6 cm high), a ladder (a small plastic white ladder, 5 cm wide and 16 cm high, having 10 steps connected to the two parallel arms, 3 cm thick), a cylinder (a black cylinder, 10 cm high and 4 cm in diameter, having a 2 cm in diameter hole on the top), a glass of steel (12 cm high and 5 cm in diameter having a black handle) and a corner (this object consisted of two gray iron pieces (10 cm sides)

with regularly pierced squares, forming a 90° angle, fixed on a quadrate 5 cm thick in sides). The corner object was used to examine the reactivity to object novelty detection changes.

2.4. Procedure

The behavioral procedure was that described in previous studies [24-26]. All animals were allowed to habituate in the experiment room (light and sound having attenuated) for at least 30 min prior to the experiments. Mice were individually placed into the empty open field for a 6 min period in order to familiarize the animal with the apparatus and to record the baseline level of locomotor activity (session 1, S1). 5 min after S1 session, mice were submitted to five successive sessions with 6 min period and 3 min interval in the open field containing the objects. The animals were returned to their home cages immediately after each session. The objects were placed in a square configuration with a central object (cone) in sessions 2-4 (S2–S4). This configuration was changed by means of transposing the cone with the cylinder in session 5 (spatial change session, S5), in such a way that the initial square arrangement was changed (objects displacement). To measure the object novelty detection memory, the glass object (as a familiar non-displaced) was replaced by the corner as a new object, at the same location (object substitution, S6 session).

2.5. Data collection and statistics

Data collection was performed using video-recordings; the observer was always blind to treatment. Two behavioral parameters were considered: locomotor activity and object exploration. Locomotor activity was recorded by counting the number of sector crossed by the animals during session 1 (S1). Object exploration was evaluated by the time spent in contact by the animals with an object during S2-S6. A contact was defined as the subject's snout actually

touching the object. To measure the spatial novelty detection, the mean time that mouse contacted with objects in S5 [both with displaced objects (DO) and non-displaced objects (NDO)] was subtracted from the mean time spent in contact with the same object in S4. In addition, to measure the object novelty detection, the mean time that the animal contacted with objects in S6 [both substituted object (SO) and non-substituted object (NSO)] was deducted from the mean time spent in contact with the same object in S5.

2.6. Drugs

The following drugs were used in the experiments: naloxone hydrochloride (μ -opioid receptor antagonist; Sigma, Poole, Dorset, UK), SCH23390 (dopamine D1-like receptor antagonist [SCH], R(1)-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-enzazepine hydrochloride; Tocris, UK), and sulpiride (dopamine D2-like receptor antagonist; Sigma, St. Louis, CA, USA); naloxone and SCH were dissolved in sterile 0.9 % saline. Sulpiride was dissolved in a minimal volume of diluted acetic acid (one drop of 5 μ l; pH 6.3), increased to a volume of 5 ml with 0.9 % physiological saline and then diluted to the required volume with a 0.9 % w/v NaCl solution. All drugs were injected intraperitoneally at a volume of 10 ml/kg.

2.7. Experimental design

Eight animals were used in each experimental group. In experiments where animals received one injection, control groups received saline or vehicle injection. Furthermore, the timing of the pre-test drug administration was 15 min before testing.

2.7.1. Experiment 1: effect of BDL on spatial and object novelty detection

In this experiment, spatial and object novelty detection were tested 2, 3 and 4 weeks after BDL and the data compared with sham animals group.

2.7.2. Experiment 2: effect of pre-test administration of naloxone, SCH23390 or sulpiride on spatial and object novelty detection in sham-operated vs. BDL mice

In this experiment, both sham- and BDL (4 weeks)-operated mice received different doses of naloxone (0.3, 0.6 and 0.9 mg/kg), SCH23390 (0.01, 0.02 and 0.04 mg/kg) or sulpiride (10, 20 and 40 mg/kg) 15 min before testing. Control groups received saline or vehicle at a volume of 10 ml/kg.

2.7.3. Experiment 3: effect of co-administration of subthreshold dose of dopaminergic agents together or each one plus naloxone on spatial and object novelty detection in sham-operated vs. BDL mice

In this experiment, 8 groups of animals were used. The animals were divided into two sets of four groups. The first four groups were sham-operated mice which received saline/saline, subthreshold dose of naloxone plus subthreshold dose of SCH23390, subthreshold dose of naloxone plus subthreshold dose of sulpiride or subthreshold dose of SCH23390 plus sulpiride. The second four groups were BDL-operated mice which received the same treatments as sham-operated mice.

2.8. Statistical analysis

Given the normality of distribution and the homogeneity of the data variance, the results were statistically evaluated by one-way ANOVA for the analysis of spatial and novelty discrimination. Further analyses for individual ‘between-group’ comparisons were carried out through post hoc Tukey’s test. In all comparisons, $P < 0.05$ represented a statistical significance.

3. Results

3.1. Effects of all interventions on locomotor activity and time of contacts

Locomotor activity analysis showed that there is no significant difference between groups in S1 section for all intervention, thus the mean $\pm$ S.E.M. has been shown in the Table 1. Moreover, similar analysis for the time of contacts indicated that there is also no any significant differences as compare to respective control, thus these results were showing in the figures without analysis representation (panels A in all Figures).

3.2. Induction of BDL

Five days after bile duct ligation, the animals showed signs of cholestasis (jaundice, dark urine and steatorrhea) which were tested qualitatively and quantitatively by [27].

3.3. Spatial and object novelty detection in BDL mice

One-way ANOVA and post hoc Tukey’s analysis revealed that 4 weeks-bile duct ligated mice decreased or increased DO [$F(3, 28) = 4.38$, $P < 0.05$] and SO [$F(3, 28) = 7.19$, $P < 0.01$] or NDO [$F(3, 28) = 9.73$, $P < 0.001$] parameters, respectively. However, none of groups altered

NSO [$F(3, 28) = 1.15, P > 0.05$] parameter. In conclusion, the data showed an impairment in both spatial and object novelty detection memories in mice following 4 weeks BDL (Fig.1).

[Fig. 1 somewhere here]

3.4. Effect of naloxone on spatial and object novelty detection in sham-operated vs. BDL mice

One-way ANOVA and post hoc analysis indicated that different doses of naloxone did not alter DO [$F(3, 28) = 2.23, P > 0.05$], NDO [$F(3, 28) = 0.61, P > 0.05$], SO [$F(3, 28) = 2.34, P > 0.05$] and NSO [$F(3, 28) = 1.07, P > 0.05$] parameters in sham-operated mice (Fig. 2, left panel). Moreover, one-way ANOVA and post hoc analysis showed that naloxone (0.3 and 0.9 mg/kg) decreased NDO [$F(3, 28) = 5.83, P < 0.01$] and increased SO [$F(3, 28) = 8.57, P < 0.001$], while did not alter DO [$F(3, 28) = 0.54, P > 0.05$] and NSO [$F(3, 28) = 1.69, P > 0.05$] parameters in BDL mice (Fig. 2, right panel). In conclusion, the data showed that naloxone restored object novelty detection memory in BDL mice.

[Fig. 2 somewhere near here]

3.5. Effect of SCH23390 on spatial and object novelty detection in sham-operated vs. BDL mice

One-way ANOVA and post hoc analysis revealed that SCH23390 decreased DO [$F(3, 28) = 3.12, P < 0.05$; at dose of 0.04 mg/kg], SO [$F(3, 28) = 31.87, P < 0.001$; at all doses] and increased NSO [$F(3, 28) = 4.96, P < 0.01$; at doses of 0.01 and 0.04 mg/kg], while did not alter NDO [$F(3, 28) = 1.53, P > 0.05$] parameters in sham-operated mice (Fig. 3, left panel). Moreover, one-way ANOVA and post hoc analysis showed that SCH23390 increased DO [$F(3, 28) = 5.62, P < 0.01$; at dose of 0.04 mg/kg] and decreased NSO [$F(3, 28) = 6.57, P < 0.01$; at dose of 0.02

mg/kg], while did not alter NDO [$F(3, 28) = 9.18, P < 0.001$] and SO [$F(3, 28) = 2.16, P > 0.05$] parameters in BDL mice (Fig. 3, right panel). In conclusion, the data showed that SCH23390 impaired both spatial and object novelty detection memories in sham-operated mice, while only restored spatial memory in BDL mice.

[Fig. 3 somewhere near here]

3.6. Effect of sulpiride on spatial and object novelty detection in sham-operated vs. BDL mice

One-way ANOVA and post hoc Tukey's analysis revealed that sulpiride decreased DO [$F(3, 28) = 3.55, P < 0.05$; at dose of 40 mg/kg], SO [$F(3, 28) = 5.09, P < 0.01$; at doses of 20 and 40 mg/kg] and increased NSO [$F(3, 28) = 3.93, P < 0.05$; at dose of 40 mg/kg], while did not alter NDO [$F(3, 28) = 1.13, P > 0.05$] parameters in sham-operated mice (Fig. 4, left panel). Moreover, one-way ANOVA and post hoc analysis showed that sulpiride increased DO [$F(3, 28) = 5.41, P < 0.01$; at dose of 20 mg/kg] and decreased SO [$F(3, 28) = 5.95, P < 0.01$; at dose of 40 mg/kg] and NSO [$F(3, 28) = 4.00, P < 0.05$; at dose of 20 mg/kg], while did not alter NDO [$F(3, 28) = 2.00, P > 0.05$] parameters in BDL mice (Fig. 4, right panel). In conclusion, the data showed that sulpiride restored spatial and potentiated object novelty detection memory deficits in BDL mice. In sham-operated mice, sulpiride impaired both memories.

[Fig. 4 somewhere near here]

3.7. Effect of co-administration of subthreshold dose of dopaminergic agents together or each one plus naloxone on spatial and non-spatial novelty detection in sham-operated vs. BDL mice

One-way ANOVA and post hoc analysis revealed that co-administration of subthreshold doses of naloxone plus sulpiride decreased DO [$F(3, 28) = 6.04, P < 0.01$] and co-administration of subthreshold doses of dopaminergic antagonists together or each one plus naloxone decreased SO [$F(3, 28) = 15.21, P < 0.001$], while no change was observed in all defined co-administration groups in NDO [$F(3, 28) = 0.62, P > 0.05$] and NSO [$F(3, 28) = 3.72, P > 0.05$] parameters in sham-operated mice (Fig. 5, left panel). Moreover, one-way ANOVA and post hoc analysis showed that only co-administration of subthreshold doses of SCH23390 plus sulpiride significantly decreased NDO [$F(3, 28) = 4.67, P < 0.01$] parameters, while none of groups did not alter DO [$F(3, 28) = 3.05, P < 0.05$], SO [$F(3, 28) = 7.21, P < 0.01$] or NSO [$F(3, 28) = 1.17, P > 0.05$] parameters in BDL mice as compared to saline/BDL group (Fig. 5, right panel). In conclusion, the data showed that co-administration of subthreshold dose of dopaminergic agents together or each one plus naloxone did not alter both memory deficits in BDL mice, while all of groups or co-administration of naloxone plus sulpiride-treated group impaired object or spatial novelty detection memory in sham-operated mice, respectively.

[Fig. 5 somewhere near here]

4. Discussion

Bile duct ligation (BDL) rodents are considered as a good experimental model for secondary biliary cirrhosis that leads to type C chronic hepatic encephalopathy (HE) [2]. The ability of these rodents to learn a passive avoidance task in a step-down or step-through apparatus is lower than that of control rodents [21, 28]. Rats with HE have also reduced ability to learn an object recognition task [29], reduced performance in associative tests [30, 31] and in working memory tests in the Morris water maze [32]. Working memory is also altered in patients with HE [33]. In accordance with other findings, our data showed that biliary obstruction after four weeks

induced a significant impairment in both spatial and object novelty detection memories. However, BDL mice did not show significant difference in locomotor activity as compared to sham-operated mice. It has been reported that rats subjected to BDL after four weeks developed biliary fibrosis which was supported by altered liver function tests, increased ammonia levels and histological staining [6, 34]. Analysis of behavior functions revealed significant impairment in cognitive and motor functions in BDL rats [6]. It seems that the disturbances of neurotransmitter systems in chronic liver failure post-BDL may be responsible for behavioral impairments observed in HE.

In the present study, pre-test intraperitoneal administration of naloxone restored object novelty detection memory deficit induced in BDL mice. Naloxone-treated groups did not alter both memories as compared to control in sham-operated mice. It has well been known that opioid receptor agonists and their antagonists affect learning and memory [35]. For example, morphine exerts amnesic effects in different models of memory [36]. The effects of morphine on memory depend on the dose and timing of drug administration [37] which can be blocked by μ -opioid antagonist, naloxone [38]. It has been reported that μ -opioidergic receptor expression in the brain of rodents changes in different time courses of BDL, so that they were down-regulated in acute BDL and up-regulated in chronic BDL model [39]. This finding confirms our previous investigations that microinjection of naloxone into the hippocampus reversed BDL-induced amnesia [19]. Our results confirm an alteration in the brain opioid neurotransmitter system in HE. Thus, it seems that in our BDL model which is a chronic BDL model, an increased sensitivity of μ -opioid receptors to the naloxone occurs. As a result, naloxone restores the impairment effect of opioids on memory in BDL mice.

Our results show that SCH23390 and the middle dose of sulpiride restored spatial novelty detection memory, while the higher dose of sulpiride potentiated object novelty detection memory in BDL mice. Moreover, the higher dose of both drugs impaired spatial novelty detection memory, while all doses of SCH23390 or two higher doses of sulpiride impaired object novelty

detection memory in sham-operated mice. In agreement with the present data there are several pieces of evidence showing that the dopamine D1 and D2 receptors are involved in the modulation of memory function [40, 41]. Moreover, the blockade of the dopamine D1 receptors by SCH23390 suppressed the learning of a single-trial passive avoidance task [41]. The amnesic response of SCH23390 may be either due to drug intrinsic activity or mediated by inhibition of a physiologic D1 receptor mechanism. To clarify this response, more experiments may be required. It has been suggested that pre-test administration of sulpiride disrupts memory by acting on pre-synaptic dopamine receptors [42]. It should be also considered that sulpiride may act both at pre- or post-synaptic D2 dopamine receptors and in turn it may release dopamine or block post-synaptic receptor subtypes. Thus the drug may alter memory retrieval differently [35, 41]. Moreover, a global decrease was observed in the dopamine content along with an increase in monoamine oxidase activity in BDL rats [6]. One may suggest that there is a cross state-dependent between the factors producing memory deficit in BDL and SCH23390. Because sulpiride acts on both pre- and post-synaptic receptors, it can induce different effects on memory impairment induced in BDL mice.

The obtained data show that subthreshold dose co-administration of dopaminergic antagonists together or each one plus naloxone did not alter both memory deficits induced in BDL mice, while all of co-administration groups or co-administration of naloxone plus sulpiride group impaired object or spatial novelty detection memory in sham-operated mice, respectively. It is suggested that opioidergic and dopaminergic systems are involved in memory deficits induced in BDL mice. However, the effect of these systems on BDL-induced memory impairment may occur through separate pathways.

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Legends

Table 1: The animals’ locomotor activity represented by the number of crossings during S1 in different treatment groups.

Fig. 1: Spatial and object novelty detection in the mice 2, 3 and 4 weeks post-surgery. Four groups of animals were used. The time spent in contact with objects in sessions 2–4 (A). Reaction to object displacement is measured as time spent in contact with either displaced (DO) or non-displaced (NDO) objects in session 5 minus the same in session 4 (B). Reaction to object substitution is measured as time spent in contact with either substituted (SO) or non-substituted (NSO) objects in session 6 minus the same in session 5). Values are expressed as mean ± S.E.M (n = 8 in each group). *P < 0.05 and **P < 0.01 different from the saline group.

Fig. 2: Effect of naloxone on spatial and object novelty detection in sham-operated vs. BDL mice. Different doses of naloxone (0.3, 0.6 and 0.9 mg/kg) or saline (1 ml/kg) were administrated 15 min before testing in the sham-operated or BDL mice. This can be followed in the left and right panels, respectively. The time spent in contact with objects in sessions 2–4 (A). Reaction to object displacement is measured as time spent in contact with either displaced (DO) or non-displaced (NDO) objects in session 5 minus the same in session 4 (B). Reaction to object substitution is measured as time spent in contact with either substituted (SO) or non-substituted (NSO) objects in session 6 minus the same in session 5). Values are expressed as mean ± S.E.M (n = 8 in each group). *P < 0.05 different from the saline group; +P < 0.05 and ++P < 0.01 different from the saline/BDL group.

Fig. 3: Effect of SCH23390 on spatial and object novelty detection in sham-operated vs. BDL mice. Different doses of SCH23390 (0.01, 0.02 and 0.04 mg/kg) or saline (1 ml/kg) were administrated 15 min before testing in the sham-operated or BDL mice. This can be followed in the left and right panels, respectively. The time spent in contact with objects in sessions 2–4 (A). Reaction to object displacement is measured as time spent in contact with either displaced (DO) or non-displaced (NDO) objects in session 5 minus the same in session 4 (B). Reaction to object substitution is measured as time spent in contact with either substituted (SO) or non-substituted (NSO) objects in session 6 minus the same in session 5). Values are expressed as mean $\pm$ S.E.M (n = 8 in each group). *P < 0.05, **P < 0.01 and ***P < 0.001 different from the saline group; ++P < 0.01 different from the saline/BDL group.

Fig. 4: Effect of sulpiride on spatial and object novelty detection in sham-operated vs. BDL mice. Different doses of sulpiride (10, 20 and 40 mg/kg) or vehicle (1 ml/kg) were administrated 15 min before testing in the sham-operated or BDL mice. This can be followed in the left and right panels, respectively. The time spent in contact with objects in sessions 2–4 (A). Reaction to object displacement is measured as time spent in contact with either displaced (DO) or non-displaced (NDO) objects in session 5 minus the same in session 4 (B). Reaction to object substitution is measured as time spent in contact with either substituted (SO) or non-substituted (NSO) objects in session 6 minus the same in session 5). Values are expressed as mean $\pm$ S.E.M (n = 8 in each group). *P < 0.05 different from the saline group; +P < 0.05 different from the saline/BDL group.

Fig. 5: Effect of co-administration of subthreshold dose of dopaminergic agents together or each one plus naloxone on spatial and non-spatial novelty detection in sham-operated vs. BDL mice. Combination of naloxone (0.3 mg/kg) plus SCH23390 (0.01 mg/kg) or sulpiride (10 mg/kg) and

combination of SCH23390 (0.01 mg/kg) plus sulpiride (10 mg/kg) were injected 15 min before testing in the sham-operated or BDL mice. Control groups received saline/saline/vehicle. This can be followed in the left and right panels, respectively. The time spent in contact with objects in sessions 2–4 (A). Reaction to object displacement is measured as time spent in contact with either displaced (DO) or non-displaced (NDO) objects in session 5 minus the same in session 4 (B). Reaction to object substitution is measured as time spent in contact with either substituted (SO) or non-substituted (NSO) objects in session 6 minus the same in session 5). Values are expressed as mean $\pm$ S.E.M (n = 8 in each group). *P < 0.05 and ***P < 0.001 different from the saline/saline/vehicle group; ++P < 0.01 different from the saline/saline/vehicle/BDL group.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Treatment	S1
Experiment 1	
Sham-operated	128.62 ± 11.15
2 weeks BDL	139.75 ± 12.53
3 weeks BDL	126.12 ± 8.58
4 weeks BDL	101.37 ± 18.75
Experiment 2	
Saline (1 ml/kg)	116.25 ± 9.89
Naloxone (0.3 mg/kg)	112.50 ± 8.43
Naloxone (0.6 mg/kg)	91.00 ± 12.64
Naloxone (0.9 mg/kg)	108.50 ± 10.45
Saline (1 ml/kg) + BDL	114.00 ± 11.90
Naloxone (0.3 mg/kg) + BDL	97.75 ± 13.62
Naloxone (0.6 mg/kg) + BDL	125.00 ± 12.05
Naloxone (0.9 mg/kg) + BDL	93.37 ± 13.02
Experiment 3	
Saline (1 ml/kg)	118.12 ± 17.32
SCH23390 (0.01 mg/kg)	96.87 ± 4.52
SCH23390 (0.02 mg/kg)	104.50 ± 11.02
SCH23390 (0.04 mg/kg)	101.00 ± 10.95
Saline (1 ml/kg) + BDL	92.37 ± 13.76
SCH23390 (0.01 mg/kg) + BDL	91.12 ± 13.24
SCH23390 (0.02 mg/kg) + BDL	87.00 ± 14.33
SCH23390 (0.04 mg/kg) + BDL	104.75 ± 13.76
Experiment 4	
Vehicle (1 ml/kg)	114.87 ± 15.17
Sulpiride (10 mg/kg)	123.37 ± 18.24
Sulpiride (20 mg/kg)	113.12 ± 10.09
Sulpiride (40 mg/kg)	126.50 ± 8.00
Vehicle (1 ml/kg) + BDL	101.37 ± 18.75
Sulpiride (10 mg/kg) + BDL	120.75 ± 19.51
Sulpiride (20 mg/kg) + BDL	112.00 ± 11.55
Sulpiride (40 mg/kg) + BDL	114.75 ± 9.92
Experiment 5	
Saline + Saline + Vehicle	123.00 ± 12.94
Naloxone (0.3) + SCH23390 (0.01) + vehicle	94.50 ± 5.79
Naloxone (0.3) + Saline + Sulpiride (10)	132.50 ± 17.02
Saline + SCH23390 (0.01) + Sulpiride (10)	94.00 ± 5.90
Saline + Saline + Vehicle + BDL	128.62 ± 11.15
Naloxone (0.3) + SCH23390 (0.01) + vehicle + BDL	101.00 ± 10.30
Naloxone (0.3) + Saline + Sulpiride (10) + BDL	91.75 ± 10.93
Saline + SCH23390 (0.01) + Sulpiride (10) + BDL	106.00 ± 12.77