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Full Length Research Paper

Effect of chronic morphine treatment on tactile learning in rat

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In our previous study, we reported that chronic morphine exposure changes neuronal response properties in rat somatosensory cortex. In this study, we investigated the effect of chronic morphine treatment on tactile learning behavior in rats. Morphine sulfate was dissolved in tap water, and was administered for 21 days. Tactile learning was assessed using the novel object recognition test (NORT) in a dark room. The chronic morphine treated group exhibited a learning impairment; the discrimination ratio was significantly lower as compared to the control group. These findings suggest that chronic exposure to morphine impaired the tactile learning in rats as the discrimination ratio was decreased following morphine administration.

Key words: Morphine, tactile learning, rat.

INTRODUCTION

The somatosensory cortex serves several important functions in the brain. By integrating and analyzing sensory information, it leads to perception of somatosensory stimuli, and by interactions with other areas in the brain, such as the striatum and motor cortex, it enables planning, execution and dynamic modulation of coordinated movement (Ferezou et al., 2007; Johansson and Cole, 1992). Cortical plasticity refers to the ability of the cerebral cortex to alter its functions as a result of experience. This ability allows us to learn new tasks, to remember past events and to recognize objects (Fox, 2002).

Opiate receptors are present in many parts of the brain including the hippocampus, and the visual and somatosensory cortices (Arvidsson et al., 1995; Lewis et al., 1983; Walker et al., 1988).

Chronic morphine usage can change normal brain function (Chieng and Williams, 1998; Pu et al., 2002; Salmanzadeh et al., 2003). The effect of morphine on brain function varies among different brain areas (Chieng and Williams, 1998; Jolas et al., 2000; Renno et al., 1992). In our previous study, we reported that chronic properties of neurons in the barrel cortex of rats. It increased the latency of neuronal responses to deflection of principal and adjacent whiskers while it had no effect on the magnitude of neuronal responses to deflection of these whiskers (Afarinesh et al., 2008).

In the present study, we investigated the effect of chronic morphine treatment on tactile learning (using the novel object recognition task) in rats. The result demonstrated that tactile learning is impaired following chronic morphine treatment.

MATERIALS AND METHODS

Animals

In this study, we used 20 male albino rats (weighting between 160 and 190 g). The animals were maintained on a 12 h/12 h light/dark cycle (lights on: 0700 to 1900 h) with free access to food and water. The animal house temperature was maintained at 23 ± 2.0°C. All experiments were conducted in accordance with standard ethical guidelines and were approved by the local ethical committee (Ethics and Animal Care Committee of Rafsanjan University of Medical Sciences).

Morphine treatment

Morphine sulfate (Temad, Iran) was dissolved in tap water, and

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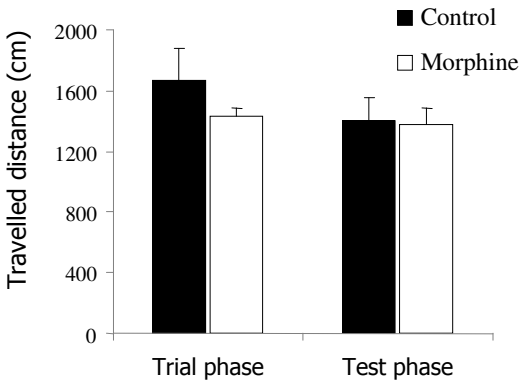

Figure 1. Activity level among control and morphine treated animals. Activity levels measured by distance travelled in 5 min during both T1 and T2 phases (Chuhan and Taukulis, 2006). All data are expressed as mean ± S.E.M. T1; training phase, T2; test phase.

approximately 20 ml of water was allotted to each rat. The concentration of morphine was as follow: 0.01 mg/ml for the first 48 h, 0.02 mg/ml for the second 48 h, 0.03 mg/ml for the third 48 h and 0.04 mg/ml for the following days. The administration of morphine was continued for 21 days. For masking the bitter flavor of morphine, sucrose (0.3 mg/ml) was added to the drinking water during the first 4 days of morphine administration (Badawy et al., 1982).

Object recognition task

The object recognition task assesses recognition memory and is based on a natural tendency of animals to preferentially explore novel objects, as opposed to familiar objects (Ennaceur and Delacour, 1988).

The experimental apparatus was a Plexiglas box (35 × 35 × 35 cm) with a black plastic floor placed in a dimly illuminated room (Howlett et al., 2004; Roozendaal et al., 2006).

The objects to be discriminated were square and triangular iron blocks. The rats' behavior was recorded by a camera positioned directly above the box and was subsequently analysed using Ethovison Software (Noldus, Wageningen, Netherlands).

The object recognition task was done in 3 phases with 24 h interval between each phase. During the habituation phase, the rats were allowed to freely explore the box in the absence of objects for 30 min. On the training day (T1), each rat was placed in the box with two identical objects and was allowed to explore for 5 min. The position and shape of the objects were changed after each animal was tested, to prevent an odor or side preference affecting the results. All rats were placed in the box at the same point and they were facing the same direction. On the test day (T2), each rat was returned to the box where it was presented with one familiar object from the training trial. The position of this object was consistent between both trials. A novel object was introduced for 5 min. Care was taken to avoid olfactory stimuli by cleaning the box and objects with 70% ethanol between rats (Aisa et al., 2007; Chuhan and Taukulis, 2006). The time spent (in seconds) exploring the objects was recorded. Exploration was defined as pointing the nose to the object at a distance □2 cm; climbing or sitting on an object was not considered as exploration. A discrimination ratio was calculated using the formula: [total time spent in exploring both objects divided by the time spent exploring novel objects only] × 100 (Chopin et al., 2002). Rats showing a total exploration time < 10 s on either training or testing were excluded (Roozendaal et al., 2006).

Statistical analysis

The statistical analysis was performed using excel and SPSS software. All data are expressed as a mean ± S.E.M. P value smaller than 0.05 has been considered as statistical significance. Differences between the groups were determined using paired t-test and t-tests.

RESULTS

Activity level

Activity levels were assessed by measuring the distance travelled during trial (T1) and test (T2) phases. In the control group, the travelled distance was in T1 (1667.8 ± 214.3 cm) and in T2 (1400.7 ± 142.2). These distances were not significantly different (Paired t-test, $t_{(5)} = 1.8$, P = 0.11). Similarly, the morphine treated group travelled 1515 ± 95.9 cm in T1 and 1380.4 ± 103 in T2. These values are not significantly different (Paired t-test, $t_{(4)} = 2.4$, P = 0.091). In the groups that received morphine, the travelled distance did not differ significantly as compared to the control group (in T1, t-test, $t_{(8)} = 0.8$, P = 0.4 and in T2, t-test, $t_{(9)} = 0.1$, P = 0.9) (Figure 1).

Object recognition task: Trial phase (T1)

The total time spent exploring both similar objects in T1 (Table 1) was not statistically significant between morphine (46.36 ± 7.5 s) and control (56.7 ± 9.5 s) groups (t-test, $t_{(9)} = 0.8$, P = 0.4). Similarly, no reliable differences were found between the two experimental groups (Table 1) for the frequency of visits to the sample objects (t-test, $t_{(9)} = 0.6$, P = 0.5).

Object recognition task: Test phase (T2)

Object exploration times for the experimental groups during the test phase (T2) are as shown in the Table 1. The mean (mean ± S.E.M) of total exploration time (in seconds) of both objects (familiar + novel) was 45.1 ± 6.9 in the control group and in the morphine treated group, it was 44 ± 9.5. These differences were not significant (t-test, $t_{(9)} = 0.09$, P = 0.9). However, in the morphine treated group, the time spent exploring a novel object was 11.9 ± 2.1 s as compared to 27.6 ± 4 s for the control group (t-test, $t_{(8)} = 2.9$, P = 0.018).

A comparison of the discrimination ratio between the two groups revealed that in the morphine treated animals, this index (36 ± 4.2%) was lesser than that of the control animals (63.2 ± 5.9%) (t-test, $t_{(8)} = 3.4$, P = 0.009) (Figure 2). These finding suggest that in the morphine treated

Table 1. Frequency and exploration times in T1 and T2 among two experimental groups.

Phase	Measured index	Control	Morphine
Trial phase (T1)	Total exploration time (s)	56.7 ± 9.5	46.36 ± 7.5
	Frequency of visits to both objects	77 ± 9.2	67.6 ± 11.3
	Time to visit familiar object	17.5 ± 3.8	28.6 ± 6.9
Test phase (T2)	Time to visit novel object	27.6 ± 4	11.9 ± 2.1*
	Total exploration time (s)	45.1 ± 6.9	44 ± 9.5
	Frequency of visits to familiar object	25.5 ± 4.4	47.8 ± 12
	Frequency of visits to novel object	40.1 ± 6.9	29.4 ± 8.3

Data are expressed as mean ± S.E.M. T2 was done 24 h after T1. *Means significant difference in time to visit novel object between control and morphine group (t-test, $t_{(8)} = 2.9$, P = 0.018).

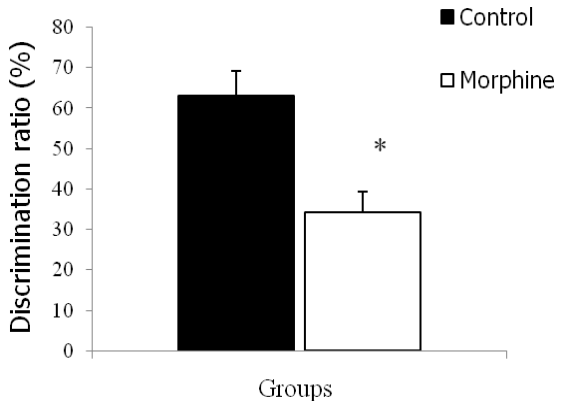

Figure 2. Effect of chronic morphine treatment on memory consolidation for object recognition task. Discrimination ratio was measured in T2 and expressed as percentage. Data are expressed as mean ± S.E.M. *) means significant difference between morphine and control groups (t-test, $t_{(8)} = 3.4$, P = 0.009).

animals the ability to discriminate between two similar and novel objects was impaired.

DISCUSSION

In our previous study, we reported that morphine dependence could change the response properties of whisker related neurons (response magnitude and latency) in the somatosensory cortex (Afarinesh et al., 2008). In this study, in line with our previous electrophysiological results (morphine changed normal neuronal response magnitude and latency to whisker movement) we demonstrated that chronic morphine exposure could also change a behavioral aspect of the somatosensory system as measured by tactile learning.

This result is in good agreement with previous studies that reported morphine impaired learning and memory. Rabbani et al. (2009) showed recognition memory impairment following induction of morphine dependence in mice. In a series of studies, it demonstrated that the administration of morphine dose and time dependently impairs retention of memory in the step-down or step-through passive avoidance learning (Rezayof et al., 2006; Zarrindast et al., 2005; Zarrindast and Rezayof, 2004).

On the contrary, recently, Soyka et al. (2010) reported a better performance for executive functions and visuo-construction in patients who were under long-term methadone treatment. Further studies are required to interpret fully, the different effect of morphine on different types of learning and memory.

The effect of morphine on sensory processing may be modulated through an extensive array of receptors and neurotransmitters. μ-opioid receptors are expressed extensively in glutamatergic pyramidal neurons and GABAergic inhibitory interneurons in the cortex and hippocampus (Arvidsson et al., 1995; Taki et al., 2000). Chronic morphine exposure can also modulate the

amount of serotonin (Tao and Auerbach, 2002), acetylcholine (Osman et al., 2005) and noradrenaline (Matsumoto et al., 1994) in the brain. These neurotransmitters modulate neural activity in the somatosensory cortex of rats (Baskerville et al., 1997; Laurent et al., 2002; Sessler et al., 1995; Upadhyaya et al., 2010). Further investigations are required to clarify the specific role of either of these neurotransmitters in mediating the effects of morphine exposure on tactile learning.

In summary, chronic exposure to morphine impaired tactile learning as measured by novel object recognition test in rats.

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