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Original article

Psychophysiological assessment of disability in male mice

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Amputation is considered as a sort of defect that its outcome is an individual's disability entailing psychological problems. The aim of present study was to examine the effects of disability on psychophysiological status of male limb-amputated mice. This experimental study was performed on 6 groups (5 male mice for each) as follows: 2 control groups (NC30 and NC60), 2 surgically amputated at right forelimb elbow (RF30 and RF60), and 2 other surgically amputated at right hindlimb knee (RH30 and RH60) groups. After 30 or 60 days, behavioral tests were performed on each group. Forced swimming test (FST), elevated plus maze (EPM), tail-pinch test (TPT), Morris water maze (MWM), and object recognition test (ORT) employed to evaluate depression, anxiety, stress, spatial memory, and learning, respectively. In FST, floating time increased significantly for RH30 group compared to NC60 group. All amputees showed a significant decrease in open arm entries in EPM compared to NC60 group. RH60 group showed a significant increase in closed arm time in EPM in comparison to NC30 group. All amputees also exhibited a significant increase in closed arm time in EPM compared to NC60 group. In sum, amputation resulted in increased anxiety and moderate impairment in spatial memory in MWM. The working memory processes in ORT and response to stressor in TPT have not altered post-amputation.

Results indicated that amputation can create some psychophysiological disorders probably due to motor behavior defects, although further studies are needed to be performed on peripheral and central effects of lower sensorimotor deprivation while following ethical rules.

1. Introduction

Organs have the most contacts with physical and natural environments and loss of a part of them severely damages an individual's mobility power and even his survival. Amputation not only causes physical damages, but also is accompanied by psychological ones (Ma'ssoumi et al., 2008). Amputation of organs includes completely or partially amputation of one or more upper limb and lower limb organs (Joghataei et al., 2002) caused by different causes including congenital physical deformity, vascular problems, cancers, incidents and accidents (Farahani¬ nia et al., 2012). Any kind of body physical deformity not only is considered a physical damage, but also entails social and psycho-emotional damages (Mousavi et al., 2004). Amputation is a life-changing incident although individuals with amputation ultimately acquire physical, social, and affective adaptations. These adaptations vary widely among different people, with majority of amputations leading to different inabilities and disabilities (Farahani nia et al., 2012). In sum, physical disability has physical and psychological aspects while being of any types and for any causes. It may create psychological and behavioral crises (Solemani Dinani et al., 2012). In their national study of devotees with bilateral upper limb amputation, Ma'soumi and colleagues, found that 41.7% of devotees had abnormal mood like depression and anxiety disorder as well as disruption of short–term memory (Ma'soumi et al., 2008). Ascherio also demonstrated that depression is common among individuals with amputations caused by mine fields (Ascherio, 1995). Stalnack and colleagues reported that psychological disorders, especially depression, have adverse effects on the life quality while chronic pains play the least role, although they are associated with disability (Stalnack et al., 2011). In a study performed to examine effects of amputation on individuals, Mosaku et al (2009) asserted that level of amputation is also a factor affecting depression and anxiety (Mosaku et al., 2009). In addition maximum progressive risk of anxiety level was observed among people with physical disability (Leger et al., 2002). In the company of advancements of medical sciences, considerable achievements have been gained in the fields of physical health preservation, mortality reduction, and increased life expectancy of disabled people. However, the individuals' mental health is one of important issues on which little research has been done. For this reason, to understand psychological factors that are effective in solving problems and behavioral abnormalities of the disabled and to make efforts in planning for their modifications and improvements, one should consider highly effective ways to prevent psychological disorders (Aslankhani et al., 2009). In present study, psychophysiological status of disabled male mice were examined and tabulated as a model.

2. Materials and methods

2.1. Study animals

This study was performed on 30 NMRI outbred mice (25±5g) obtained from Animal Breeding Center of School of Veterinary Medicine, Razi University, Kermanshah, Iran. Animals were kept under conditions of 12 hr of light and 12 hr of dark, at temperature range of 23±2°C and with water and food ad libitum. During experiments, ethical rules related to working with laboratory animals were followed. Animals were assigned randomly to 6 groups (n=5 for each) as follows:

1) NC30 control group: no surgical operation was performed on them; after 30 days, they were tested behaviorally.

2) NC60 control group: no surgical operation was performed on them; after 60 days, they were tested behaviorally.

3) RF30 group with amputated right forelimb at elbow joint: they were tested behaviorally after 30 days.

4) RF60 group with amputated right forelimb at the same region as group 3: they were tested behaviorally after 60 days.

5) RH30 group with amputated right hindlimb at knee joint: they were tested behaviorally after 30 days.

6) RH60 group with amputated right hindlimb at the same region as group 5: they were tested behaviorally after 60 days.

2.2. Procedure of amputation surgery

All animals, except NC30 and NC60 control groups, were anesthetized intraperitoneally by using ketamine-xylazine cocktail. Amputation surgery was done on elbow joint of right forelimbs of animals in groups 3 and 4 as follows: Initially, fur over target region was shaved and then sterilized and cleaned by alcohol. Then, 0.1 ml of lidocaine (2%) was injected subcutaneously into area above surgical region. In order to prevent bleeding, forelimbs were ligated on area above elbow joint which was amputated after removing its skin. After that, amputated region was sutured and immediately sterilized with povidone-iodine (10%) solution. Then, warm normal saline solution 0.9% (10 ml/kg) in amount of 0.1 ml was intraperitoneally injected. Also, animals received 0.2 ml of cefazolin to prevent postoperative infection. Having been conscious fully, amputated animals were injected intraperitoneally by 0.1 ml of tramadol to manage postoperative pain. After performing surgical operation, all animals were kept in a warm place for 24 hr. Then, 0.5 ml of acetaminophen syrup has been poured into drinking bottles of amputees for 4-5 days. For animal groups 5 and 6, surgical amputation was performed on right hindlimbs at knee joints. Then, all necessary actions were taken as described above. All animals were monitored post operatively until they recovered. After 30 or 60 days, behavioral tests were carried out on each group. Forced swimming test (FST), elevated plus maze (EPM), tail-pinch test (TPT), Morris water maze (MWM), and object recognition test (ORT) used for evaluating depression, anxiety, stress, spatial memory, and learning abilities of mice, respectively as described elsewhere (Ivani et al., 2013).

2.3. Statistical analyses

SPSS version 18 software was used to analyze data. One-way variance analysis (ANOVA) and post hoc Tukey's HSD test were used to see if there are significant differences among groups. The times of finding hidden platform in different trials of MWM were analyzed using a repeated-measure ANOVA, followed by a post hoc Tukey's HSD test. Data were expressed as the mean ± SEM and p value < 0.05

3. Results

In FST, results of floating time indicated that RH30 group exhibited a significant increase in this parameter as compared to that of NC60 group. Droppings pellets of group RH60 showed a significantly higher increase than those of RF30 and RF60 groups. Due to increased dropping pellets as anxiety index, RH60 group was more anxious (Table 1).

Table 1
The effects of amputation on floating time, an index of depression, in forced swimming test in male mice.

*Groups1(n=5)	Floating time (Sec)	Dropping pellet
NC30	84.00 ± 56.298	0.80 ± 0.447
NC60	33.20 ± 27.770	1.00 ±1.224
RF30	109.00 ± 41.659	0.20 ± 0.447
RF60	73.60 ± 50.742	0.20c ± 0.447
RH30	137.00 ± 19.924#	1.000 ± 0.000
RH60	69.25 ± 66.605	2.00 ± 0.816

In EPM, it was observed that open arm entries, an index of decreased anxiety, of RF30, RF60, RH30 and RH60 groups showed a significant decrease as compared to that of group NC60. For close arm entries, an index of increased anxiety, no significant differences were observed among groups (P>0.05). For closed arm time, RH30

[*] NC30 and NC60: day 30 and 60 normal control. *RF30 and RF60: day 30 and 60 right forelimb amputees. *RH30 and RH60: day 30 and 60 right hindlimb amputees. Date is shown as Mean ± S.E.M. * and # show significant difference compared with NC30 and N60, respectively.

group indicated an increase in comparison with group NC30 which was significant at P<0.05. Also RH30, RH60, RF30 and RF60 groups showed an increase compared to group NC60 which was significant at P<0.05. For open arm time, NC30 group exhibited a decrease in comparison with NC60 group (P<0.05). In addition RF30, RF60, RH30 and RH60 groups showed a decrease in this parameter compared to group NC60 (P<0.05; Table 2).

Table 2
The effects of amputation on anxiety status in elevated plus maze trials in male mice.

†*Groups (n=5)	CE (sec)	OE (sec)	CT (sec)	OT (sec)
NC30	10.60 ± 1.816	5.60 ± 2.302	156.20 ± 37.171a	55.60 ± 30.038#
NC60	7.20 ± 1.923	9.20 ± 4.604	88.20 ± 40.702b	119.80 ± 42.144*
RF30	7.60 ± 2.888	1.40 ± 2.190#	229.20 ± 42.008#	13.60 ± 20.767#
RF60	9.00 ± 0.707	2.60 ± 2.701#	189.20 ± 51.683#	29.40 ± 52.609#
RH30	7.80 ± 3.346	2.60 ± 1.341#	230.60 ± 33.291#	15.60 ± 11.886#
RH60	6.50 ± 2.645	2.50 ± 1.914#	245.00 ± 22.553#	9.50 ± 7.141#

Results regarding to the time of biting response, an index of stress, were not significant at P>0.05 among groups but dropping pellets of NC30 and RH30 groups are significant at P<0.05 (Table 3).

Table 3
The effects of amputation on duration of biting response in tail-pinch test in male mice.

**Group(n=5)	Time of biting response (sec)	Droppings pellet
NC30	189.75 ± 112.571	3.40 ± .894a
NC60	146.00 ± 111.764	1.20 ± 1.643
RF30	232.75 ± 69.777	2.00 ± 1.870
RF60	282.25 ± 11.324	1.00 ± 1.224
RH30	174.80 ± 123.786	0.20 ± .447*
RH60	202.33 ± 131.970	2.00 ± 2.160

Time needed to find a hidden platform in lesser than 60 sec has been considered as an index of spatial memory enhancement that was not significantly different among studied group in MWM (Table 4).

Table 4
The effects of amputation on time (< 60 sec) of finding a hidden platform in Morris water maze in male mice.

***Group	Day1 (Sec)	Day2 (Sec)	Day3 (Sec)	Day4 (Sec)	Day5 (Sec)
NC30	20.93 ± 6.107	18.77 ± 10.459	10.12 ± 4.125	9.37 ± 1.050	17.01 ± 4.568
NC60	17.45 ± 7.691	16.30 ± 6.042	10.72 ± 4.860	14.11 ± 7.199	8.33 ± 3.184
RF30	19.62±4.772	20.75 ± 8.539	24.81 ± 11.689	24.60 ± 20.107	21.71±11.986
RF60	21.87 ± 9.454	15.20 ± 8.296	18.83 ± 3.752	22.14 ± 14.884	26.45 ± 21.757
RH30	14.50 ±8.046	30.00 ± 0	32.00 ± 16.970	0.00 ± 0.000	0.00 ± 0.000
RH60	13.66 ± 6.658	15.00 ± 0	27.00 ± 0	27.00 ± 5.656	30.75 ± 0

Results of time to finding a hidden platform upper than 60 sec on day 4 showed an increase for RH30 group compared to NC30 and NC60 groups (P<0.05). Also RH30 group indicated an increase in this parameter in comparison with RF30 and RF60 groups (P<0.05; Table 5). On day 5 or target trial for time > 60 sec to find a hidden

[*] NC30 and NC60: day 30 and 60 normal control. *RF30 and RF60: day 30 and 60 right forelimb amputees. *RH30 and RH60: day 30 and 60 right hindlimb amputees. Date is shown as Mean ± S.E.M. * and # show significant difference compared with NC30 and N60, respectively. CE: Close arm entries; OE: Open arm entries; CT: Close arm time; OT: Open arm time.

**NC30 and NC60: day 30 and 60 normal control. *RF30 and RF60: day 30 and 60 right forelimb amputees. *RH30 and RH60: day 30 and 60 right hindlimb amputees. Date is shown as Mean ± S.E.M. * and # show significant difference compared with NC30 and N60, respectively.

***NC30 and NC60: day 30 and 60 normal control. *RF30 and RF60: day 30 and 60 right forelimb amputees. *RH30 and RH60: day 30 and 60 right hindlimb amputees. Date is shown as Mean ± S.E.M. * and # show significant difference compared with NC30 and N60, respectively platform, RF60, RH30 and RH60 groups showed an increase compared to NC30 group (P<0.05; Table 5). Group RF30 showed a decrease in this parameter in comparison with RH30 and RH60 groups (P<0.05; Table 5).

Table 5
The effects of amputation on time (> 60 sec) of finding a hidden platform in Morris water maze in male mice.

‡Group	Day1 (Sec)	Day2 (Sec)	Day3 (Sec)	Day4 (Sec)	Day5 (Sec)
NC30	3.00± 1.414	1.00 ± .000	1.00 ± 0.000	1.00 ± 0.000	1.00 ± .000a
NC60	1.33 ± .577	0.00 ± 0.000	2.00 ± 0.000	1.00 ± 0.000	0.00 ± 0.000
RF30	3.50 ±1.000	2.80 ± 1.095	3.00 ± 1.414	2.00 ± 1.4140	2.00± 1.4140
RF60	2.80 ± .836	2.60 ± .547	2.75 ± 1.500	1.75 ± 0.957	3.00± 1.414*
RH30	3.00±1.000	3.80 ± .447	3.60 ± .894	4.00 ± .000ab*	4.00 ± 0.000
RH60	2.75 ± .957	3.75 ± .500	4.00 ± .000	3.25 ± 0.957	4.00 ± 0.000

ORT results were not significant different for both time of old object exploration, an index of amnesia, and time of new object recognition, an index of working memory enhancement (P>0.05; Table 6). Dropping pellets of RH60 group were higher than NC30 and NC60 groups which was significant at P<0.05. In addition RH60 group showed an increase compared to RH30 group (P<0.05). Due to increased dropping pellets as anxiety index, RH60 group was more anxious (Table 6).

Table 6
The effects of amputation on time of detecting objects in learning object recognition test in male mice.

Group (n=5)	Old object Recognition Time (Sec)	New object Recognition Time (Sec)	Droppings pellet
NC30	126.20 ± 46.948	128.00 ± 12.747	0.20 ± 0.447
NC60	94.60 ± 42.193	116.20 ± 43.286	0.20 ± 0.447
RF30	143.80 ± 22.420	152.00 ± 8.154	1.00 ± 0.707
RF60	102.80 ± 29.609	130.80 ± 16.543	0.80 ± 1.095
RH30	132.40 ± 45.500	116.20 ± 54.421	0.20 ± 0.447
RH60	16.010±150.50	126.65 ± 29.341	2.00 ± 1.414*#

4. Discussion

Amputation is considered as one of causes of disability occurrence (Esquenazi, 2000) that affects social adaptation and mental health and resulting disability imposes more psychological pressure on physically disabled people than on other ordinary and normal ones (Moradi et al., 2011). Therefore, present study was performed to examine psychophysiological effects of disability on male mice.

Forced swimming valid test was used to evaluate depression in animal models. Conventionally, floating time, which is the very discontinuation of limb motions, was considered in this test as an index of despair and depression. Increasing time of floating is an important index of animal's depression. Results from this study, showed that forelimb-amputated day-30 and 60 animal groups were not different in floating times. But hindlimb-amputated day-30 animal groups showed an increase in floating time compared to healthy day-60 ones. In this line, Hawamdeh et al (2008) demonstrated that individuals with amputated lower limbs experienced high levels of depression and anxiety (Hawamdeh et al., 2008). Our results showed that with an increase in time passed after amputation, hindlimb-amputated day-60 animals exhibited no changes in floating times. So it appeared that with increased time after amputation, animals' depression decreased partially. In addition Desmondm (2007) showed that level of depression decreased with an increase in time passed after amputation and over time (Desmondm,

‡ NC30 and NC60: day 30 and 60 normal control. *RF30 and RF60: day 30 and 60 right forelimb amputees. *RH30 and RH60: day 30 and 60 right hindlimb amputees. Date is shown as Mean ± S.E.M. * and # show significant difference compared with NC30 and N60, respectively

** NC30 and NC60: day 30 and 60 normal control. *RF30 and RF60: day 30 and 60 right forelimb amputees. *RH30 and RH60: day 30 and 60 right hindlimb amputees. Date is shown as Mean ± S.E.M. * and # show significant difference compared with NC30 and N60, respectively.

2007). In this study, animals' droppings, an excitatory factor, was considered as a physiological index based on previous investigations (e.g., Pardon, 2000). This indicated that all forelimb-amputated day-30 and-60 animals as well as those hindlimb day-60 ones experienced some kinds of depression. In this sense, different levels of amputation could make a difference in floating times. Khademi et al (2012) demonstrated that amputated individuals were involved in depression with different degrees (Khademi et al., 2012). Since depression is a multidimensional problem in which several factors are involved, here amputation – related disability can play a role in causing depression.

Elevated plus maze test was used to examine anxiety in disabled mouse model. As with typical evaluation of this test, anxiety was evaluated by avoidance of open arms. Results from this study showed that all amputees showed a decrease in the number of entries and in time spent on open arms compared to healthy control animals. No significant difference was observed among study animal groups in terms of the number of entries to closed arms. But time spent on closed arms showed an increase for day-30 hindlimb-amputees compared to day-30 healthy ones. Also, an increase was observed for all forelimb- and hindlimb- amputated animals in both day-30 and day-60 in comparison to 60-day healthy animals. This indicated that healthy animals exhibited differently anxiety behaviors. However, amputated animals experienced more anxious behaviors than healthy ones. As observed, all amputated animals did not experience different anxious behaviors in 30- and 60-day of evaluation, but all of them suffered anxiety with the same degree. This shows that disability with whatever type and cause leads to anxiety that needs more investigations. Brenes et al (2005) showed that anxiety relates to development of disability dependently (Brenes et al 2005). Our study showed that amputation-induced disability contributes to anxiety among male mice.

Tail-pinch test was employed to evaluate stress paradigm, in which the behavior associated with non-masticatory muscle activity of jaws was the only one taken into account and recorded collectively during the test. According to the results from TPT, no significant difference was observed among study groups. Animal droppings have taken in experiment as a physiological index of stress-induced motility changes in gastrointestinal tract. Results showed that dropping excretion (fecal pellet numbers) by healthy animals of day-30 was different from that of hindlimb-amputated animals on day-30. Droppings is an excitatory factor (Pardon, 2000) which can be indicative of that due to more excreted droppings, healthy animals probably experienced more stress during 1st month post-amputation. In a study, Abeyasinghe et al (2012) showed that prevalence of post-traumatic stress disorder was more among soldiers with lower and upper body amputations and with spinal cord damages (Abeyasinghe et al 2012). Copurqlu et al (2010) also demonstrated that traumatic amputation is one of important causes of acute stress disorder and post-traumatic stress disorder (Copurqlu et al., 2010). Our results stated that all amputated animals experienced some kinds of stress in TPT that requested further investigations.

In order to examine animal's spatial memory, MWM test was employed in this study. Evaluation of this test was considered on the basis of delay time in finding a hidden platform within and after 60 sec. Results showed that no significant difference was observed among groups in terms of delay time in finding a hidden platform within 60 sec from 1st to 5th day of test. This indicated that all amputated animals probably suffered same degree of memory disorder. For time >60 sec of finding a hidden platform on 5th or target day, 30-day forelimb-amputated animal and both 30- and 60-day hindlimb-amputated ones showed an increase in comparison with 30-day healthy animals. For time >60 sec of finding a hidden platform, forelimb-amputated animals of 30-day group showed a decrease compared to 30- and 60-day hindlimb-amputated animals. Ultimately, on day 5, hindlimb-amputated animals showed more reduction of memory than forelimb-amputated ones. This finding indicates that difference in amputated organs, right forelimb versus hindlimb, could make a difference in animals' working memory that needs more experiments. To our knowledge there is not a comparable study in the literature.

Object recognition test was used to evaluate learning abilities in mice. This test was based on total time during which animals could explore old and new objects in an open field. Results indicated that no difference was observed among groups in terms of approaching and exploring old and new objects. However dropping excretion, as the index of anxiety, showed an increase for hindlimb amputated animals of 60-day group compared to healthy animals of 30-day and 60-day groups. This was the case for the former in comparison with hindlimb-amputated animals of 30-day group. This shows that dropping excretion was more for hindlimb-amputated animals of 60-day therefore this group was more anxious. Based on our findings, working memory trend in ORT was disturbed for all amputated animals compared to control ones. Our results suggest that examination of disabled mouse model should be performed on a larger population and in longer time if ethics permits us

5. Conclusions and recommendations

Results of this study indicated that amputation could cause psychophysiological disorders the major reason for which is impairment of animals' motor behavior. Although further studies are needed to be performed on the peripheral and central aspects of lower sensorimotor deprivation, it is recommended that:

1) Psychophysiological endpoints would be studied with disabled left forelimb and hindlimb models;

2) Based on the role of time in changing behaviors, psychophysiological endpoints would be examined with disabled left forelimb and hindlimb models during longer periods of time; and finally

3) Given that behaviors are various, psychological status would be studied with the aid of other behavioral test.

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