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A history of chronic morphine exposure during adolescence increases despair-like behaviour and strain-dependently promotes sociability in abstinent adult mice

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Abstract

A crucial issue in treating opiate addiction, a chronic relapsing disorder, is to maintain a drug-free abstinent state. Prolonged abstinence associates with mood disorders, strongly contributing to relapse. In particular, substance use disorders occurring during adolescence predispose to depression later in adulthood. Using our established mouse model of opiate abstinence, we characterized emotional consequences into adulthood of morphine exposure during adolescence. Our results indicate that morphine treatment in adolescent mice has no effect on anxiety-like behaviours in adult mice, after abstinence. In contrast, morphine treatment during adolescence increases behavioural despair in adult mice. We also show that morphine exposure strain-dependently enhances sociability in adult mice. Additional research will be required to understand where and how morphine acts during brain maturation to affect emotional and social behaviours into adulthood.

Keywords

Morphine; adolescence; abstinence; sociability; depression; anxiety

1. Introduction

Addiction, defined mainly by a loss of control over drug seeking and consumption [1], is a chronic relapsing disorder. A crucial issue in treating addiction is to maintain a drug-free abstinent state. Prolonged abstinence is characterized by a negative affective state [2] and retrospective epidemiological studies consistently show that substance use disorders, notably for opiates, strongly associate with mood disorders [3–6]. Accordingly, a history of opiate dependence confers a 50% lifetime risk of depressive comorbidity [7]. Although recognized

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Conflicts of interest

All authors report no biomedical financial interest or potential conflicts of interest.

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as a major contributor to relapse, even after decades of abstinence [8–11], depression has received little attention in preclinical models of opiate dependence. Rodent studies have shown that during prolonged withdrawal from chronic opiate treatment, i.e. abstinence, drug seeking is potentiated by drug-associated cues [12–14], while motivation toward natural reinforcers is disrupted [15–19]. Only a few studies explored emotional behaviours, showing that depressive-like behaviours emerge during opiate withdrawal, from 3–6 days [20, 21] up to 2–3 weeks in rat [22] and after 9 days in mouse [23]. We established a mouse model of opiate protracted withdrawal [24], in which depressive-like behaviours strengthen during extended abstinence. Following chronic exposure to escalating doses of morphine, a prototypical opiate, abstinent mice progressively exhibited increased despair-like behaviour and low sociability. Importantly, both deficits were reversed by chronic antidepressant treatment.

Adolescence, the transition from child- to adulthood, is a plastic and dynamic process that largely condition psychopathological risk over the lifespan [25–27], notably the emergence of mood disorders [28]. Adolescents frequently experience recreational properties of drugs of abuse [27] which, in some individuals, eventually leads to substance use disorders. Longitudinal studies reveal that substance use disorders occurring during adolescence predispose to depression later in adulthood [29–31]. In rodent models, a few studies have addressed effects of opiates in adolescent individuals. In self-administration paradigms, opiate reinforcement is increased for morphine in rats [32] and oxycodone in mice [33], while unchanged for heroin in rats [34]. Further, after a short (3 days) spontaneous withdrawal from chronic morphine treatment, adolescent mice showed decreased despair-like behaviour, while no such effect is found in adults [23]. These increased reinforcing and decreased withdrawal-induced aversive properties of opiates in adolescent rodents are coherent with increased drug initiation during this period in human [35, 36]. To our knowledge, the effects of chronic opiate exposure during adolescence on emotional responses in the adult have not been addressed in mice so far.

In the present study, we used our mouse model to characterize emotional consequences of a morphine exposure during adolescence in adult mice. Adolescent mice received escalating morphine treatment during 6 days. After 4 weeks of abstinence, we assessed emotional-like behaviours in these mice that had become adults. We performed this analysis in three classically used inbred strains: C57BL/6JCrI (B16J, as in our previous work [24]), C57BL/6NCrI (B16N) and Balb/cByJCrI (Balb).

2. Material and methods

2.1 Animals

Postnatal day 21 (P21) male mice were obtained from Charles Rivers Laboratories (St-Germainsur-l'Arbresle, France) and habituated to housing conditions (4 animals per cage; 12h light/dark cycle; food and water available *ad libitum*) for 1 week. All experimental procedures were performed according to standard ethical guidelines (European Community Guidelines on the Care and Use of Laboratory Animals 86/609/EEC).

2.2 Drugs

Morphine sulfate (Francopia, Gentilly, France) was prepared in saline solution (0.9% sodium chloride) and injected at a volume of 10 ml/kg.

2.3 Experimental procedure

Age of mice for morphine treatment was chosen based on previous studies (see [23] and [27] for a review) so that adolescent mice received morphine injections from P30 to P35. We

used the same morphine regimen as in our previous study in adult [24]. Our laboratory has previously shown that this regimen induces a strong physical dependence [37], one of the diagnosis criteria for opiate addiction in human [38]. Mice were administered intraperitoneally (i.p.) twice daily with (i) escalating doses of morphine (20, 40, 60, 80, 100 mg/kg, n=12/mouse strain) for 5 days, followed by a single 100 mg/kg injection on day 6, or (ii) saline solution as a control (n=12/mouse strain). Each housing cage contained only saline-treated or only morphine-treated mice. The animals were then maintained drug free for 4 weeks (see Fig. 1A for experimental timeline). As an index of general opiate effects, mice were weighed daily during chronic injections and after 10 days (P45) and 29 days (or 4 weeks, P64) of abstinence. Beginning at P64, when abstinent mice have become adult, emotional-like and social behaviours were evaluated every other day (except for open-field and social interactions) using classical paradigms in the following order (see below) [24, 39]. For further information on behavioural testing procedures, see http://www.ics-mci.fr/service_neurobiology_behaviour_tests.html.

2.4 Behavioural testing

2.4.1 Light-Dark (LD)—The apparatus consisted of two identical boxes (20x20x14cm) interconnected with a dark tunnel (5x7x10cm, Imetronic, Pessac, France) equipped with infrared sensors. The light compartment (white walls) was brightly illuminated (1900 lux) from the ceiling. Mice were placed in the dark compartment (black walls) and the latency to enter the lit compartment, as well as the time spent in each compartment, were recorded during 5 min.

2.4.2 Open-field (OF)—Mice were placed during 30 min in 44x44 cm open-field arenas (Panlab, Barcelona, Spain), fitted with infrared beams allowing automated locomotor activity measures (Actitrack, Barcelona, Spain). Arenas were indirectly lit at 150 lux. Distance and time spent in the centre were recorded as locomotor activity and anxiety measures. This exposition to the open-field also served as habituation to the environment of the social interaction test, performed on the following day.

2.4.3 Social interactions (SI)—Pairs of unfamiliar mice, from different home cages but of similar treatment condition and weight, were placed simultaneously for 10 min in the open-field arena, indirectly lit at 50 lux. Our previous work indicates that both prior habituation to the arena and dim lighting favour social interactions in poorly anxiogenic conditions [24]. Using an ethological keyboard, we measured the number of occurrences and the total duration of social interaction behaviours (sniffing, following and pawing contact).

2.4.4 Tail suspension (TS)—Mice were suspended by the tail 50 cm above the floor. Activity was automatically monitored during the last 4 min of the 6-minute test (MED associates Inc, St Albans, USA), as previously described [39], with a threshold defining immobility behaviour. Latency to the first immobilization was also noted.

2.4.5 Forced swim (FS)—Mice were placed for 6 min into a glass cylinder (height, 27 cm; diameter, 18 cm) filled with 3.5 liters of water (23±1°C), and immobility time was recorded during the last 4 min [39] by direct observation using an ethological keyboard. Latency to the first immobilization was also noted.

2.4.6 Y-maze (YM)—Mice were individually placed in a Y-shaped apparatus consisting in three arms (placed at 120° from each other), and allowed to move freely (continuous procedure) for 5 min under moderate lighting conditions (100 lux in the centre-most region). An arm entry was counted when the mouse had all four paws inside the arm. The sequence of successive entries into the three arms was scored for each mouse, and the Y-maze

performance, i.e. the percentage of spontaneous alternation performance, was defined as the ratio of actual alternations to possible alternations $(\text{total arm entries} - 2) \times 100$ [40, 41].

2.5 Statistical analysis

All data are expressed as mean \pm sem. Statistical analysis was performed using two-way analysis of variance (ANOVA) with strain and treatment as independent variables. For the weight analysis, we used a three-way mixed ANOVA, with strain and treatment as between-subject factors and time as within-subject factor. When required multiple group comparisons were performed using Fischer's post-hoc analysis. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Weight growth

A growth spurt is a hallmark of adolescence in rodents, with a rapid increase in body weight during the first 5 weeks of life [27]. Accordingly, ANOVA revealed a significant main effect of time [$F(7,455)=1408$; $p < 0.0001$], confirming that mice progressively gained weight during the course of the study, from P30 to P64 (Fig. 1B). ANOVA also revealed significant main effects of strain [$F(2,65)=9.99$; $p=0.0002$] and morphine treatment [$F(1,65)=49.5$; $p < 0.0001$], but no interaction between these 2 factors [$F(2,65)=0.78$; $p=0.46$]. Post-hoc comparisons showed that morphine-treated mice show reduced body weight compared to saline controls between P31 and P35 (post-hoc; $p < 0.0001$). Balb mice showed decreased body weight, compared to B16J or B16N (post-hoc; P31–35, $p < 0.05$) during the course of chronic injections.

During abstinence, while left drug-free in their home-cages, morphine-abstinent mice gained more weight than saline controls (time x treatment interaction, [$F(7, 43)=19.2$; $p < 0.0001$]). Thus, after 4 weeks of abstinence and prior to behavioural testing, morphine abstinent mice recovered body weight no longer different from saline controls (post-hoc; P45, $p < 0.0001$; P64, $p=0.07$).

3.2 Light-dark

Results for every behavioural test are summarized in Table 1. We first analysed the anxiety-like behaviour of morphine- and saline-treated mice in the light dark test. Analysis of the percentage time spent in the aversive brightly lit compartment (Fig. 2A) revealed a significant main effect of strain [$F(2,65)=5.6$; $p=0.0056$], but no effect of morphine treatment [$F(1,65)=2.31$; $p=0.13$] and no interaction [$F(2,65)=0.04$; $p=0.96$]. Inter-strain comparisons showed that the B16N showed highest levels of anxiety, an effect significant against B16J mice ($p=0.0014$) and very close to significance against Balb mice ($p=0.051$). We did not find any difference ($p=0.20$) between B16J and Balb mice in this test, coherent with previous results [42].

Accordingly, latency to first entry into the lit compartment (Fig. 2B) was significantly different across strains [$F(2,65)=19$; $p < 0.0001$]. Morphine treatment had no effect on this parameter [$F(1,65)=0.17$; $p=0.68$], and there was no interaction [$F(2,65)=0.90$; $p=0.41$]. Inter-strain comparisons indicated that latency was increased in B16N mice compared to both Balb ($p < 0.0001$) and B16J mice ($p < 0.0001$).

3.3 Open-field

We also measured the percentage time spent in the centre of the OF (Fig. 2C), an index of anxiety-like behaviour in rodents. We found a significant effect of strain [$F(2,65)=4.42$; $p=0.016$], with no effect of treatment [$F(2,65)=2.37$; $p=0.13$] and no interaction

[F(2,65)=0.02; p=0.98]. Inter-strain comparisons indicated that Balb mice showed increased anxiety-like behaviour as compared to B16J (p=0.007), in agreement with previous data [43, 44]. Balb mice were also more anxious than B16N (p=0.02), while B16J and B16N strains did not differ (p=0.70), as already reported [45].

We also assessed locomotor activity, as measured by the distance travelled in the OF (Fig. 2D). Two-way ANOVA revealed a significant effect of strain [F(2,65)=5.81; p=0.005], with no effect of morphine treatment [F(1,65)=1.46; p=0.23] and no interaction [F(2,65)=1.78; p=0.18]. Coherent with results from other groups [44, 46], Balb mice exhibited lower locomotor activity as compared to B16J (post-hoc, p=0.001) and B16N mice (post-hoc, p=0.04). The 2 later strains showed similar locomotor activity (post-hoc, p=0.20).

3.4 Social interactions

We assessed social interactions after 4 weeks of abstinence by measuring the duration and number of occurrences of social behaviours in the three mouse strains. ANOVA showed that strain had a significant effect on the time pairs of unfamiliar mice spent interacting (Fig. 3A) [F(2,29)=7.19; p=0.003], with Balb mice showing lower levels of sociability compared to B16N (p=0.026) and B16J (p=0.0005) mice. In addition, morphine treatment had a significant effect on the duration of social behaviours [F(1,29)=4.58; p=0.04], and there was a significant interaction between strain and treatment [F(2,29)=4.14; p=0.026]. Post-hoc comparisons showed that morphine abstinence strongly increased the duration of social behaviours in B16N (p=0.031) and Balb (p=0.018) mice, but had no effect in B16J mice (p=0.28). In B16N and Balb mice, increased duration of social behaviours was not due to a general hyperactivity, as locomotor activity was not modified in morphine abstinent mice (see Fig. 2D).

We also counted the number of social behaviours (Fig. 3B). ANOVA revealed that, similar to the analysis of duration, strain had a significant effect [F(2,29)=7.2; p=0.0028] on these behaviours. In addition, morphine treatment had a significant effect [F(1,29)=5.7; p=0.024], while there was no interaction [F(2,29)=1.8; p=0.18].

3.5 Tail suspension (TS) & forced swim (FS)

We assessed the duration of immobility in the TS and FS tests, as indexes of despair-like behaviours. In both TS [F(2,65)=6.44; p=0.003] and FS [F(2,65)=4.3; p=0.018], we found a significant main effect of strain on duration of immobility (Fig. 4A & 4C). Balb mice exhibited the higher levels of immobility, being significantly different from B16N (p=0.011) and B16J (p=0.019) in the FS test (Fig. 4C), and from B16N (p=0.0008), but not B16J (p=0.17), in the TS test (Fig. 4A). Comparing B16J and B16N strains, we found that they show similar despair-like behaviour in the FS test (p=0.84), while immobility is decreased in B16N in the TS test (p=0.034).

Importantly, statistical analysis revealed that morphine abstinence significantly increased the time spent immobile in the FS test [F(2,65)=4.01; p=0.049], an effect close to significance in the TS test [F(2,65)=3.56; p=0.063]. Since morphine had no effect on locomotion (see OF, in Fig. 2D), we interpret this increased immobility in abstinent animals as increased behavioural despair. We found no interaction between morphine and strain in both tests (TS, [F(2,65)=0.40; p=0.67] and FS, [F(2,65)=0.50; p=0.61]), although visual inspection of the FS data suggest that increased despair-like behaviour in morphine abstinence mainly occurs in B16N mice.

We also measured latency to the first immobilisation (Fig. 4B & 4D), another index of despair-like behaviour [39]. We found a significant effect of strain in the TS (Fig. 4B) [F(2,65)=4.7; p=0.012] and in the FS (Fig. 4D) [F(2,65)=21.9; p<0.0001] tests. Coherent

with the strain effect on immobility duration, Balb mice showed faster immobilization than (i) B16J in FS ($p < 0.0001$) and TS ($p = 0.048$), and (ii) B16N in FS ($p < 0.0001$) and TS ($p = 0.003$). B16N and B16J strains showed similar latencies in TS ($p = 0.17$) and FS ($p = 0.99$) tests. ANOVA also revealed a significant effect of morphine treatment on latency to first immobilization in the FS [$F(1,65) = 6.4$; $p = 0.013$], but not in the TS [$F(1,65) = 0.19$; $p = 0.67$] tests. There was no interaction between the 2 factors (TS, [$F(2,65) = 1.0$; $p = 0.37$] and FS, [$F(2,65) = 0.7$; $p = 0.49$]), although the data suggest that decreased latency to immobilization mainly occurs in Balb mice.

3.6 Y-maze

Morphine abstinent mice were tested using the Y-Maze (Table 1) to assess spatial working memory [47]. ANOVA revealed a significant effect of strain [$F(2,64) = 4.71$; $p = 0.012$], with no effect of morphine exposure [$F(1,64) = 0.26$; $p = 0.61$] and no interaction [$F(2,64) = 0.63$; $p = 0.53$]. Balb mice showed decreased spontaneous alternation (SPA) as compared to B16J and B16N mice (post-hoc, $p = 0.007$ and $p = 0.013$ respectively). This was already reported for B16J mice [41].

4. Discussion

In human, adolescent substance use disorders associate with an increased risk for anxiety and depression throughout life [29–31]. In the present study we used our mouse model of opiate abstinence to assess effects of chronic morphine exposure during adolescence on emotional responses in the adult. This model only partially recapitulates the human condition as it is based on passive drug exposure. In this respect it will be important to examine long-term effects of morphine across adolescence and adulthood in rodent models involving voluntary drug intake.

Opiate addiction [48, 49], as well as anxiety and depressive disorders [50], are strongly determined by genetic factors. Inbred mouse strains allow studying these factors in preclinical models [51, 52] and high inter-strain variability has been reported for opiate physical dependence [53, 54], locomotor sensitization [55] and reinforcement [55, 56]. Few studies, however, have explored opiate-induced emotional responses across different mice strains. Here, we compared three different mouse strains. We found that abstinence from chronic morphine treatment increases the expression of despair-like behaviours and modifies sociability. Further, we observed that these effects are better detected in B16N and Balb strains.

Weight growth in adolescent mice is stopped during chronic morphine treatment

We measured body weights during escalating morphine treatment and abstinence, as an index of general opiate effects. In our previous study [24], morphine treatment decreased body weight by about 10% in adult mice, while no weight change occurred in saline-treated controls. In this study, morphine prevented adolescent growth, which otherwise occurred in saline treated animals. This effect was transient and differences were no longer significant after 4 weeks of abstinence. Morphine was shown to regulate feeding behaviours and locomotor activity in adult mice [57]. These morphine activities may also operate in adolescent animals and contribute to the observed growth arrest in our study.

Inbred mice strains show strong behavioural differences

Following 4 weeks of abstinence, we assessed several behavioural measures of emotion-like responses in adult mice. First, our results reveal higher levels of anxiety in B16N compared to B16J in the LD, but not in the OF. To our knowledge, only one study has compared both strains in these 2 tests. Matsuo *et al* [58]) reported (i) in the OF, increased anxiety-like

behaviour in B16N as compared to B16J mice and (ii) in the LD, no difference between the 2 strains. Taken together, the results by Matsuo et al and our data suggest that B16N mice exhibit higher levels of anxiety-like behaviours than B16J across different paradigms, yet the detection of these subtle effects highly relies on experimental conditions. In the OF, Balb mice presented higher levels of anxiety compared to the 2 other strains. Taken together, results from the LD and OF indicate that the 2 tests, using different aversive stimuli, measure different constructs of anxiety-like behaviours [59–61].

We also found that adult Balb mice exhibit low sociability compared to B16N and B16J. Using a three-chambered apparatus, previous studies showed that Balb mice exhibit poor exploration of the compartment containing an engaged congener [62, 63]. Our results further indicate that direct physical interactions in freely moving animals are also lower in Balb mice.

Finally, we found that adult Balb mice show higher levels of despair-like behaviours compared to B16J and B16N in TS and FS tests. Previous studies comparing Balb and B16J mice failed to detect increased levels of behavioural despair in Balb mice in the FS [64], and found no change [65] or a small increase [66] in the TS. The detection of strain differences in these paradigms therefore likely relies on experimental conditions. Overall, these results show that we detected phenotypic variations in our experimental conditions across inbred mice strains, both coherent with and extending on previous findings.

Chronic morphine treatment during adolescence does not affect anxiety-like responses and working memory in adulthood

Epidemiological studies in human indicate that opiate addiction strongly associates with an increased lifetime risk for anxiety disorders [7]. Our results in mice however suggest that adolescent morphine exposure does not disrupt the expression of anxiety-related responses later in adulthood, at least in two behavioural paradigms (LD and OF tests). Previous studies in our laboratory reported no effect of opiate abstinence in the same tests of anxiety, following adult exposure to either morphine [24] or heroin (Lutz et al, unpublished). Available data from other groups have yielded variable results, and reported either increased [67, 68] or decreased [67, 69] anxiety-like behaviour. Altogether, effects of repeated opiate exposure on anxiety-like behaviours appear complex, and highly depend on the species, opiate doses, duration of withdrawal and behavioural measures. Therefore, the comorbidity between opiate addiction and anxiety remains difficult to address in rodent models.

Opiate dependence is also associated with deficits in working memory [70, 71]. We tested the effect of adolescent morphine treatment on the cognitive performance of adult animals in a task of spatial working memory. While previous reports indicate that opiate exposure acutely impairs rodents performance in similar maze tasks [72–75], our present results suggest that these effects do not persist during abstinence.

Chronic morphine treatment during adolescence regulates social behaviours

Intriguingly, we also observed that exposure to morphine in adolescent mice enhances social interactions in the adults. Numerous data document the crucial regulation of social behaviours by the mu opioid receptor, the molecular target of morphine [37], across various developmental stages and species. This receptor regulates early social bonds to parents or caregivers in mice [76], primates [77] and humans [78]. Later in life, social interactions with peers play an important adaptive role during adolescence [79], promoting both encounters with individuals outside the natal group and independence from this group. Interestingly, endogenous opioid peptides are released throughout the brain during social behaviours in adolescent rodents [80, 81], and the mu receptor tightly regulates social reward both in

adolescent rats [82] and mice [83]. In addition, acute social isolation enhances social investigation upon retrieval of a familiar congener in adolescent mice (P25–45), an effect that increases with the duration of the isolation period [84]. Acute morphine treatment blocks this effect of social isolation, suggesting that morphine administration can substitute for social reinforcement. Altogether these data show that the opioid system plays a critical role in the developmental maturation of social functions. Here, we show that chronic morphine treatment in adolescent mice increases social behaviours in adulthood. Considering the phylogenetic conservation of behavioural hallmarks of adolescence, and their regulation by the opioid system, we hypothesize that repeated exposure to morphine in adolescent mice could sensitize neurological substrates responsible for the expression of social behaviours over the lifespan. Interestingly, Van den Berg et al showed that morphine treatment in adolescent rats [81], combined with social isolation, increases social interactions later in adulthood. Also, activation of the mu opioid receptor within the nucleus accumbens has been shown recently to promote social play [85] in adolescent rats. We may speculate that in adolescent mice, chronic morphine treatment potentiates rewarding properties of encounters with cage-mates through opioid-sensitive circuits, possibly within the nucleus accumbens. This effect may be long-lasting and account for increased sociability expressed at adult age. Interestingly, in the aforementioned model of social investigation following isolation [84], a higher sensitivity to morphine was found in adolescent Balb mice as compared to B16J mice, a result that parallels those from the present study, showing pro-social, long-term morphine effects in Balb but not in B16J mice. The implication in such long-term morphine effects of neuropeptides regulating social behaviours [86], and sensitive to exogenous mu receptor activation (such as oxytocin [87] and vasopressin [88]) remains to be determined.

Together with our previous study [24], we show in B16J mice that morphine exposure during adulthood, but not during adolescence, decreases sociability after an abstinence period. Thus, in this mouse strain, activation of the mu opioid receptor at different developmental stages produces divergent long-term effects on sociability. In the future, unravelling similar ontogenic changes in the regulation of social behaviours may help explain how these behaviours fulfil different functions (peer relationships during adolescence or reproduction in adulthood, for example) across development.

Chronic morphine treatment during adolescence enhances despair-like behaviours in adulthood

Adolescent morphine treatment globally increased (across the 3 strains) the expression of despair-like behaviours at adult age, as revealed in the FS. This finding suggests that inbred mice strains may be used to model opiate misuse in human adolescents in relation to the lifetime risk of depression, and could help identify underlying molecular adaptations. In adult mice [24], abstinence from chronic morphine treatment also increased despair-like behaviour, although the effect was detected in the TS, but not in the FS, suggesting that the abstinence phenotype may differ across developmental ages, possibly through divergent opioid mechanisms. In adults, we showed that chronic morphine exposure impairs the serotonergic neurotransmission [24, 89]. Future studies should explore whether morphine similarly affects serotonin neurons in adolescent mice.

In B16J mice, our results show that morphine exposure during adolescence had no effect on levels of adult sociability or despair-like behaviour in the TS, with a modest effect in the FS. As already mentioned, our previous work indicated that abstinence from morphine exposure at the adult age produces a robust depressive-like syndrome, with increased behavioural despair and low sociability that can be prevented by chronic antidepressant treatment [24]. Together, our two studies suggest that adolescent B16J mice, when compared with adult mice, are less susceptible to morphine-induced emotional dysfunction. Interestingly,

Hodgson et al recently reported that 3 days after chronic morphine exposure, adolescent but not adult C57BL/6 mice exhibit decreased immobility in the FS [23]. The authors interpreted decreased immobility during adolescent acute withdrawal as mood elation and suggested that “opioid withdrawal might affect mood differentially across ages”. Within this line, beyond acute withdrawal, adolescence also associates with decreased sensitivity to long-term morphine-induced mood deregulation. Therefore, in adolescent mice, decreased severity of withdrawal, already reported for other drugs of abuse (see [90] for a review), may contribute to decreased effects of prolonged abstinence.

Pharmacokinetic differences between adolescent and adult B16J mice might account for such age-related differences in morphine effects. However, this hypothesis is unlikely, as a recent report has shown that upon systemic injection (i.p.; 17,8 mg/kg [91]) morphine achieves comparable plasma and brain levels from adolescence to adulthood. Alternatively, we suggest that the pharmacodynamic properties of morphine may vary during the lifespan. Adolescent and adult B16J mice may show different levels of expression or functional activities of the mu opioid receptor, whereby chronic exogenous mu receptor activation may lead to long-term depressive-like deficits in adult mice only. The mu receptor shows during rat development dynamic region-specific patterns of expression. After birth, expression of the mu receptor slowly declines during at least 24 months in the pituitary gland [92], while rapidly increasing in the hippocampus [93] and the striatum [94], two structures regulating emotions. Ontogenic variations have also been reported in MOR regulation of monoamine release [95], activity of the HPA axis [96] and prolactin secretion [97]. In mice, two early studies suggested that the expression of the mu opioid receptor globally increases during post-natal development in the whole brain [98, 99]. Additional studies will be required to correlate developmental behavioural adaptations and post-natal regulation of mu receptor expression and function in brain regions regulating emotional-like behaviours.

5. Conclusion

In conclusion we show that, as for adult mice, morphine exposure in adolescent mice increases behavioural despair after prolonged abstinence, providing an animal model for comorbidity between opiate addiction and depression throughout the animal lifespan. We also show that, in contrast to adult mice, morphine treatment during adolescence enhances sociability in adulthood. Additional research will be required to understand where and how morphine acts during brain maturation to affect in the long-term emotional and social behaviours.

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Research Highlights

- Chronic morphine treatment in adolescent mice does not affect anxiety-like responses into adulthood
- Chronic morphine treatment in adolescent mice increases despair-like behaviour into adulthood
- Chronic morphine treatment in adolescent mice strain-dependently enhances sociability into adulthood

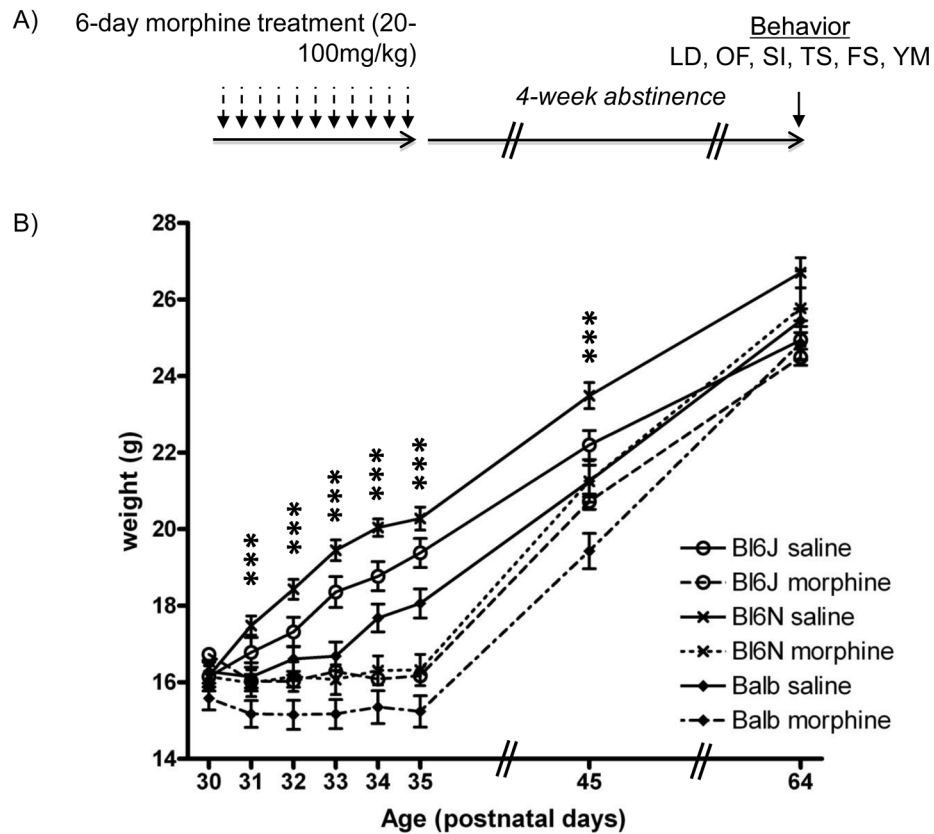


Figure 1.

(A) Experimental timeline. (B) Weight evolution during chronic morphine treatment and 4-week abstinence. C57BL/6J (B16J), C57BL/6N (B16N) and Balb/cByJ (Balb) mice received chronic injections with increasing morphine doses (20–100 mg/kg, i.p., twice daily), or saline (n=12 mice/group/strain), during 6 days, from post-natal day 30 (P30) to P35. After 4 weeks of abstinence, behavioural responses were assessed in the following order: light-dark (LD), open-field (OF), social interactions (SI), tail suspension (TS), forced swim (FS) and Y-maze (YM) tests. As an index of general opiate effects, mice were weighed daily during chronic injections and after 10 (P45) and 29 (P64) days of abstinence. Morphine-treated mice showed significantly decreased body weights compared to saline controls from P31 to P45. Balb mice showed lowest weight across the experiment (see text). Data are mean \pm s.e.m. *** $p < 0.001$, ANOVA main effect of morphine treatment.

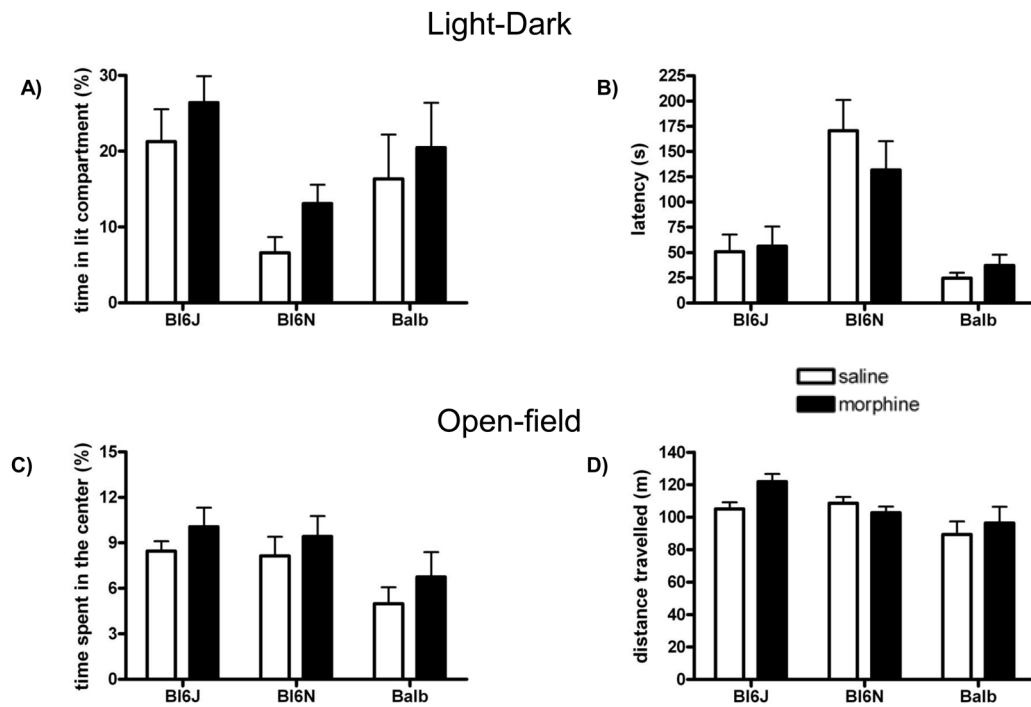


Figure 2. Chronic morphine treatment during adolescence does not affect anxiety-like responses and locomotor activity in adulthood

4 weeks after the last morphine or saline injection (n=12 mice/group/strain), we assessed behavioural responses in C57BL/6J (B16J), C57BL/6N (B16N) and Balb/cByJ (Balb) mice. Morphine abstinence had no effect on anxiety-like behaviours in the Light-Dark (**A**, **B**) and the Open-Field (**C**), or on locomotor activity in the Open-Field (**D**). Significant effects of strain are not depicted (see text). Data are mean \pm s.e.m.

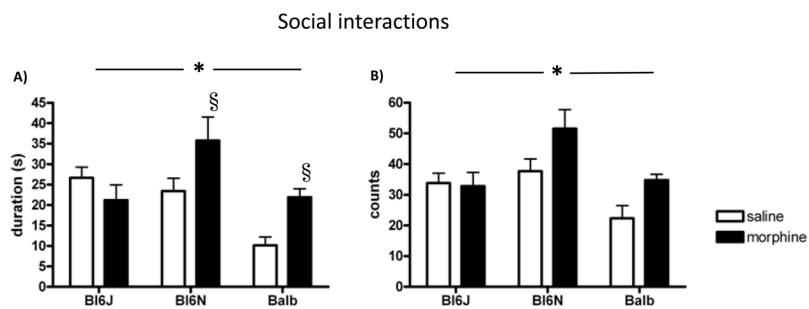
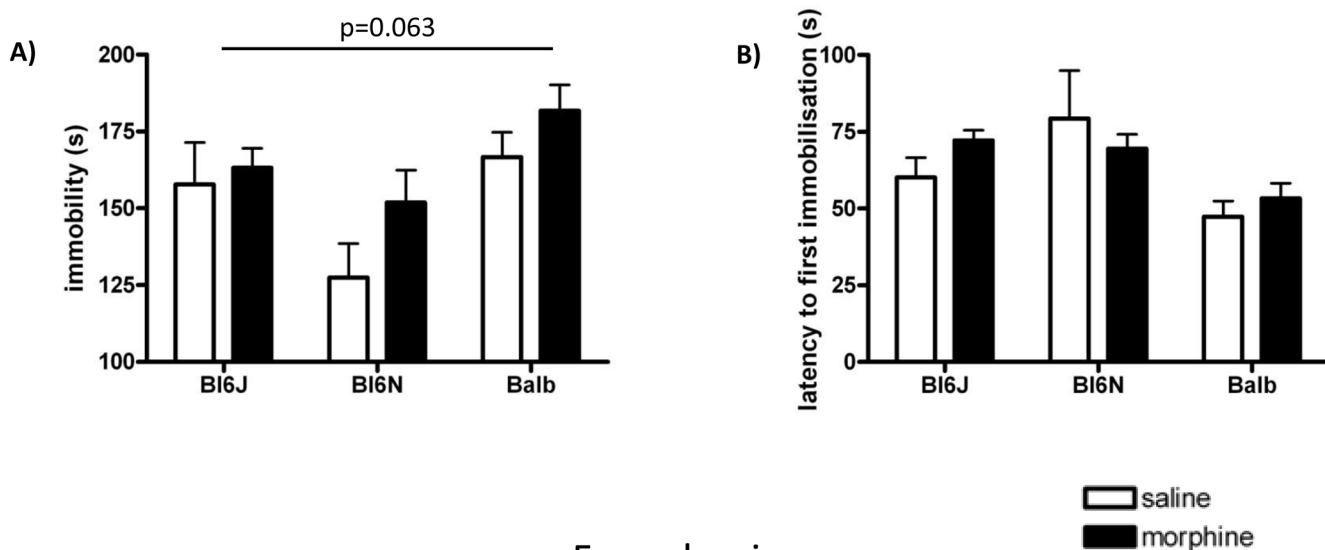


Figure 3. Chronic morphine treatment during adolescence increases social behaviours in adulthood

4 weeks after the last morphine or saline injection (n=12 mice/group/strain), we assessed social interactions in C57BL/6J (B16J), C57BL/6N (B16N) and Balb/cByJ (Balb) mice. Morphine abstinence increased the number of occurrences (A) and the total duration (B) of social behaviours in B16N and Balb mice, but not in B16J. Significant effects of strain are not depicted (see text). Data are mean \pm s.e.m. * $p < 0.05$, ANOVA main effect of morphine abstinence; § $p < 0.05$, post-hoc comparisons for the effect of morphine abstinence in each strain.

Tail suspension



Forced swim

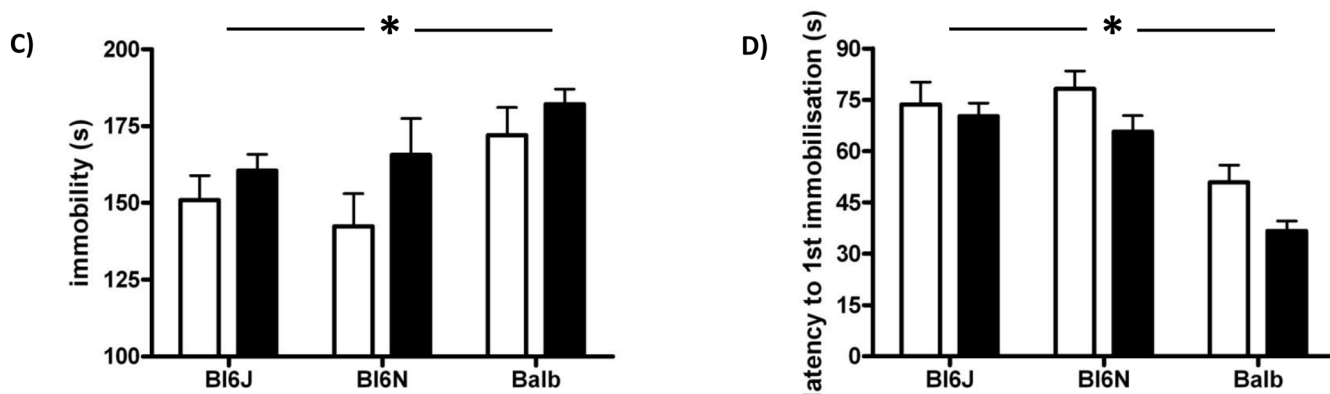


Figure 4. Chronic morphine treatment during adolescence increases despair-like behaviours in adulthood

4 weeks after the last morphine or saline injection (n=12 mice/group/strain), we assessed behavioural responses in tail suspension and forced swim tests of C57BL/6J (B16J), C57BL/6N (B16N) and Balb/cByJ (Balb) mice. Time spent immobile (**A and C**) and latency to first immobilization (**B and D**) are represented. Morphine abstinence increased despair-like behaviours in the forced swim (immobility and latency to first immobilization, **C and D**), and this effect was almost significant in the tail suspension (immobility duration, **A**). Significant effects of strain are not depicted (see text). Data are mean \pm s.e.m. * $p < 0.05$, ANOVA main effect of morphine abstinence.

Summary of the effects of chronic morphine treatment during adolescence on anxiety-like behaviours (light-dark, LD, and open-field, OF), social interactions (SI), despair-like behaviours (tail suspension, TS, and forced swim, FS) and spatial working memory (Y-Maze, YM), measured 4 weeks after the last morphine (n=12 mice/strain) or saline injection (n=12 mice/strain) in C57BL/6J (B16J), C57BL/6N (B16N) and Balb/cByJ (Balb) mice. Data are mean \pm s.e.m.

Table 1

Test	Parameter	B16J			B16N			Balb			Stats (p values)		
		Saline	Morphine	Saline	Morphine	Saline	Morphine	Saline	Morphine	Strain	Morphine	Interaction	
LD	time in lit compartment (%)	21.3 \pm 4.3	26.4 \pm 3.5	6.6 \pm 2.1	13.1 \pm 2.5	16.3 \pm 5.9	20.5 \pm 5.9	0.0056	0.13	0.96			
	latency to enter the lit compartment (s)	50.8 \pm 16.9	56.5 \pm 19.4	170.6 \pm 30.5	131.6 \pm 28.6	24.7 \pm 5.1	37.2 \pm 10.5	<0.0001	0.68	0.41			
OF	time spent in the centre (%)	8.5 \pm 0.6	10.1 \pm 1.3	8.2 \pm 1.2	9.4 \pm 1.3	5.0 \pm 1.1	6.7 \pm 1.6	0.016	0.13	0.98			
	distance travelled (m)	105.1 \pm 4.0	121.9 \pm 4.8	108.6 \pm 3.7	102.7 \pm 3.8	89.3 \pm 8.1	96.4 \pm 9.9	0.005	0.23	0.18			
SI	duration (s)	26.7 \pm 2.6	21.2 \pm 3.8	23.4 \pm 3.1	35.8 \pm 5.8	10.2 \pm 2.1	21.9 \pm 2.1	0.003	0.04	0.026			
	counts	33.8 \pm 3.2	32.8 \pm 4.4	37.7 \pm 4.0	51.5 \pm 6.3	22.3 \pm 4.2	34.8 \pm 1.9	0.0028	0.024	0.18			
TS	immobility (s)	157.8 \pm 13.7	163.1 \pm 6.4	127.3 \pm 10.4	151.8 \pm 9.4	166.7 \pm 8.0	181.7 \pm 8.5	0.003	0.063	0.67			
	latency to 1 st immobilization (s)	60.1 \pm 6.4	72.2 \pm 3.4	79.3 \pm 15.7	69.6 \pm 4.6	47.3 \pm 5.2	53.3 \pm 4.9	0.012	0.67	0.37			
FS	immobility (s)	151.0 \pm 7.8	160.6 \pm 5.2	142.4 \pm 10.7	165.6 \pm 11.8	172.2 \pm 8.9	182.1 \pm 4.9	0.018	0.049	0.61			
	latency to 1 st immobilization (s)	73.6 \pm 6.6	70.2 \pm 3.8	78.2 \pm 5.2	65.7 \pm 4.7	50.9 \pm 4.9	36.6 \pm 2.9	<0.0001	0.013	0.49			
YM	spontaneous alternation (%)	64.7 \pm 3.5	58.8 \pm 2.9	61.7 \pm 3.3	60.1 \pm 3.5	49.9 \pm 4.6	52.6 \pm 4.7	0.012	0.61	0.53			